2016

The Expression of Dopamine-Related Genes and Behavioral Performance in Mice

Victoria Lynne Dershem
Wright State University

THE EXPRESSION OF DOPAMINE-RELATED GENES AND BEHAVIORAL PERFORMANCE IN MICE

A thesis submitted in partial fulfillment of the
requirements for the degree of
Master of Science

By

VICTORIA LYNNE DERSHEM
B.S. Forensic Science, Eastern Kentucky University, 2014

2016
Wright State University

WRIGHT STATE UNIVERSITY

GRADUATE SCHOOL

December 19, 2016

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Victoria Lynne Dershem ENTITLED The Expression of Dopamine-Related Genes and Behavioral Performance in Mice BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

Ryan D. Jankord, Ph.D.
Thesis Co-director

Nicholas V. Reo, Ph.D.
Thesis Co-director

Madhavi P. Kadakia, Ph.D.
Department Chair

Committee on
Final Examination

Ryan D. Jankord, Ph.D.

I. Michael Leffak, Ph.D.

Nicholas V. Reo, Ph.D.

Robert E.W. Fyffe, Ph.D.
Vice President for Research and
Dean of the Graduate School

ABSTRACT

Dershem, Victoria Lynne. M.S., Department of Biochemistry and Molecular Biology, Wright State University, 2016. The Expression of Dopamine-Related Genes and Behavioral Performance in Mice.

While the neurotransmitter dopamine has been well-studied for its role in mood regulation and activation of the intrinsic reward pathway, several psychiatric disorders linked to dopamine are also known to cause memory impairment, a phenomenon which has attracted much less attention. In the current study, whole-transcriptome RNA sequencing was performed, and transcript levels of several dopamine-related genes were compared to results of behavioral assays designed to test spatial and emotional memory, as well as anxiety. The results suggest a positive relationship between expression level of *Nurr1*, a nuclear receptor known to initiate transcription of genes necessary for dopaminergic signaling, and both emotional and spatial memory. However, no correlation was observed between expression of tyrosine hydroxylase, dopamine transporter, or any variant of dopamine receptor, and any of the behavioral results. These results are consistent with previous research findings that *Nurr1* plays a role in memory consolidation, and suggests a dopamine-independent regulation.

TABLE OF CONTENTS

LIST OF FIGURES

Figure **Page**

LIST OF TABLES

Table **Page**

ACKNOWLEDGMENTS

Firstly, I would like to extend my undying gratitude to my PI, Dr. Ryan Jankord, my adviser within the department, Dr. John Paietta, and my committee members, Drs. Michael Leffak and Nick Reo, for their mentorship and guidance in overcoming the many hurdles that presented themselves over the course of my research. I am also grateful to my former mentor, the late Dr. Heather Hostetler, for broadening my horizons and instilling in me an interest in signaling proteomics, despite my resistance.

I also extend my appreciation to all the members of the Applied Neuroscience group for welcoming me into their group with open arms, and tolerating my steep learning curve as I attempted neuroscience for the first time – especially Dr. Milene Brownlow, Ben Holmes, Naomi Bechmann, and Raquel Moore, without whom none of this work could have been accomplished.

And finally, thank you to my parents, Linda and Les; my grandparents, Lou and Sue; and my best friends, Dani Lawson and Ken Miller. You all encouraged me when I thought I couldn't go on with my studies. We've laughed, we've cried. You let me think out loud at you, even if you didn't understand a word of it. I love you all. Everyone named on this page is important, but I would absolutely not have gotten to this point without your unwavering support.

This research was supported in part by an appointment to the Student Research

Participation Program at the U.S. Air Force Research Laboratory, 711th Human Performance Wing, Airman Systems Directorate, Warfighter Interface Division, Applied Neuroscience Branch administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and USAFRL.

This thesis is dedicated to the loving memories of Louis Anthony Tamburino, Ph.D. and David Robert Jones.

I. INTRODUCTION

Purpose

According to the National Institute of Mental Health, 18.1% of adults and 46.3% of children suffered from a mental illness in 2014. In the same year, over 42,000 lives were lost to suicide in the United States alone, making it the country's tenth leading cause of death (Kochanek, Murphy, Xu, & Tejada-Vera*.,* 2016). Furthermore, despite countless advances in psychiatric research in recent years, the suicide rate has climbed from 10.5 to 13 per 100,000 individuals since 1999 (Curtin, Warner, & Hedegaard*,* 2016). It has been demonstrated that chronic stress can contribute to development of psychiatric disorders, although we are far from fully understanding the biochemical mechanisms by which this occurs. Many psychiatric disorders are believed to involve dysregulation of dopamine signaling, and therefore, there is tremendous interest in the biochemical causes of these disorders. This is a broad field, however, as dopamine signaling is regulated by a diverse range of proteins. These include enzymes involved in catecholamine synthesis, transporters involved in regulation of synaptic neurotransmitter concentrations, and receptors which intercept synaptic dopamine and translate the messenger signal into intracellular cascades which lead to transcription, translation, or post-translational modification which modulate activity of fully-formed proteins.

Many conditions listed in the *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition* that are associated with dopamine, such as Major Depressive

Disorder and schizophrenia, feature symptoms relating to impaired or altered ability to consolidate or access long-term memory (American Psychiatric Association, 2013).

While dopamine has been highly studied in regards to mood disturbances, less emphasis has been placed on the mechanisms by which the pathophysiology of these disorders affects memory formation and/or retrieval. Because of this, the current study focuses on dopaminergic signaling in the hippocampus, a neural structure which is closely associated with declarative—including emotional and spatial—memory (Duvernoy, 2005). By expanding our knowledge of the causes behind memory-related psychiatric symptoms, the current study may assist in furthering our knowledge of how to treat them.

For this study, critical genes from various aspects of dopaminergic regulation (Fig. 1) were examined: tyrosine hydroxylase (TH, *Th*), which converts tyrosine into L-DOPA, the precursor to dopamine; the dopamine transporter (DAT, *Dat1*), which facilitates reuptake of unbound synaptic dopamine; the five subtypes of dopamine receptor (both excitatory and inhibitory); and NURR1 (*Nurr1*), a nuclear receptor which transcriptionally activates both *TH* and *Dat1*. Using RNA sequencing and behavioral assays, the current study sought to determine whether or not mRNA expression levels of these genes in the hippocampus relate to various measures of memory and emotion after exposure to a chronic stress environment. By studying the relationship between dopamine-signaling related gene expression and behaviors related to memory and fear, insight may be gained into the genetic pathologies of diseases that affect these cognitive functions. By narrowing down which genes are most crucial in these pathways, they can

then be further investigated in pharmaceutical and other clinical studies.

Dopamine

Dopamine (3,4-Dihydroxyphenethylamine) is an organic biochemical messenger that was first synthesized *in vitro* by George Barger and James Ewens in 1910 (Fahn, 2008). Arvin Carlsson (who would eventually win the 2000 Nobel Prize in Medicine or Physiology for his discoveries) determined that not only is dopamine a precursor to other catecholamines, but is a neurotransmitter in its own right (Fahn, 2008). It has since been discovered that dopamine plays a role in many aspects of neural activity, including intrinsic reward, decision making, behavior, and physical movement (Schultz, 2007). Upon release of dopamine into the synapse, it accomplishes these functions by binding dedicated receptors on the post-synaptic neuron. There are two distinct classes of dopamine receptor, both of which are G protein-coupled receptors: the D_1-like and the D_2-like. The D_1-like group consists of DRD1 and DRD5, which activate adenyl cyclase by coupling to the $G\alpha_s$ protein, while the D_2-like group, comprised of DRD2, DRD3, and DRD4, inhibit adenyl cyclase by way of the $G\alpha_i$ or $G\alpha_o$ proteins (Beaulieu & Gainetdinov, 2011). According to Rondou, Haegeman, and Van Craenenbroeck (2010), the affinities of the dopamine receptors, as measured by K_i values, are approximately 2500 nM for DRD1, 500 nM for DRD2, 20-100 nM for DRD3, 43-400 nM for DRD4 (depending on allelic variations), and 225 nM for DRD5. Together, these receptors modulate the conversion of the neurotransmitter signal into intracellular signaling cascades, which can either facilitate or inhibit the phosphorylation of CREB and resulting gene transcription (Fig. 2).

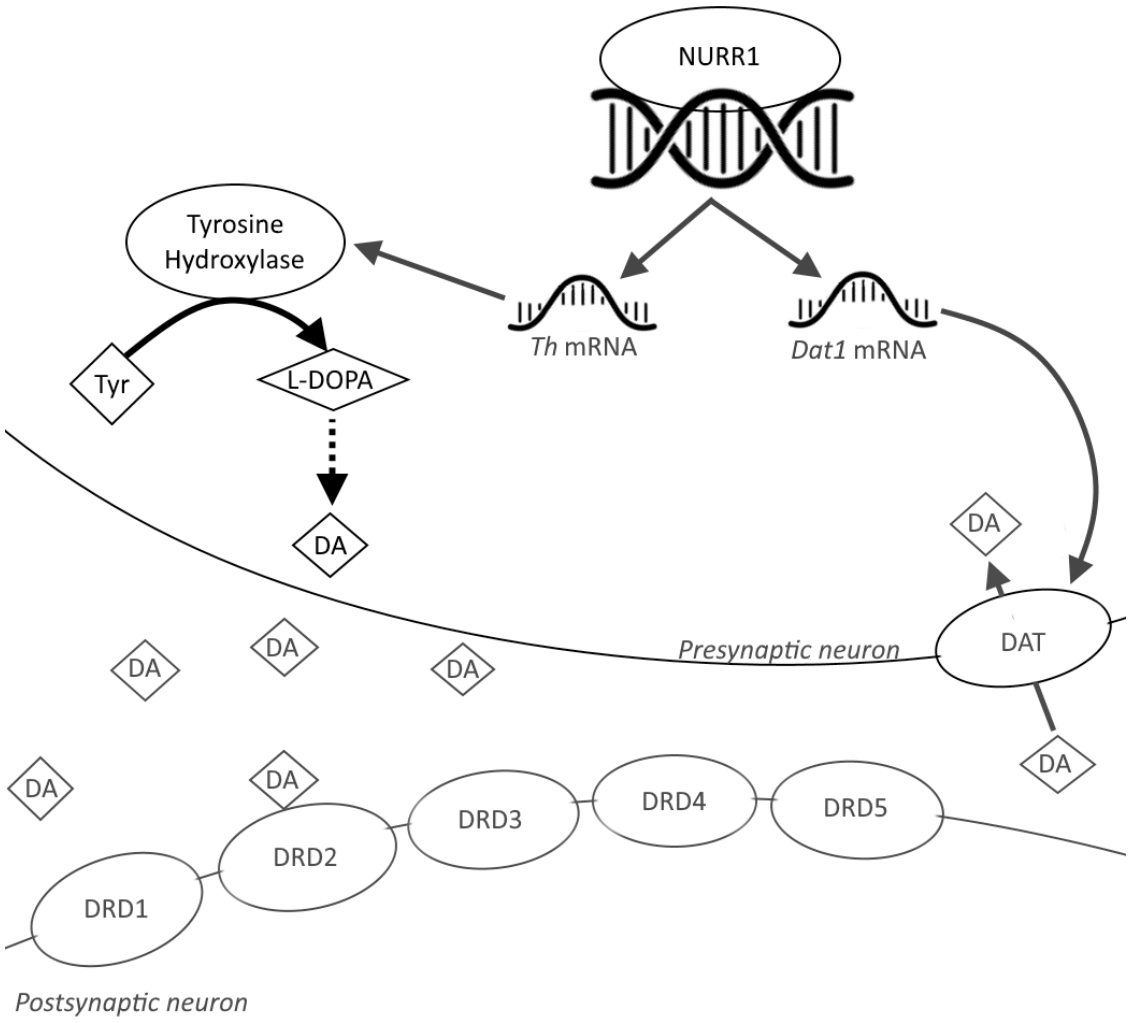

Figure 1: Roles of genes examined in the present study. NURR1, a nuclear receptor, is a transcriptional activator of dopamine-related genes, such as Th and Dat1. TH converts tyrosine to L-DOPA as part of the catecholamine synthesis pathway. DAT regulates synaptic dopamine concentration by facilitating reuptake of DA into the presynaptic neuron. Finally, the five subtypes of dopamine receptor (DRD1-5) are found in the membrane of the postsynaptic neuron and bind DA that has been released into the synapse.

The first aspect of the current study was to explore how subjects' mRNA levels of the studied genes (including dopamine receptors) varied across the population, despite identical handling and stress protocols. At least two studies have demonstrated a decrease in extraneuronal dopamine levels in response to stress (Ahmad, Rasheed, Banu, & Palit, 2010; Rasheed *et al.*, 2010), and there is evidence to suggest that dopamine receptor expression is inversely proportional to synaptic dopamine concentrations. With this in mind, it is important to understand the processes involved in the catecholaminergic synthesis pathway and release of DA into the synapse.

The precursor to all catecholamines is the amino acid tyrosine, which goesthrough a number of enzymatic alterations to reach its final state(s). Tyrosine is first converted to L-DOPA by TH, and then to dopamine by L-aromatic amino acid decarboxylase (AADC). Alternately, a second, minor pathway exists in which tyrosine undergoes decarboxylation and is converted into tyramine (another amino acid) before being hydroxylated to dopamine by CYP2D6 (Meiser, Weindl, & Hiller, 2013; Hiroi, Imaoka, & Funae, 1998). In dopaminergic neurons, this step is the endpoint. However, in (nor)adrenergic neurons, further steps are necessary. Dopamine is converted to norepinephrine by dopamine-β-hydroxylase (DBH; Ciaranello, Wooten, & Axelrod, 1976), and then, as appropriate, to epinephrine by phenylethanolamine N-methyltransferase (PNMT) (Sabban & Kvetňanský, 2001). These pathways are illustrated in Fig. 3.

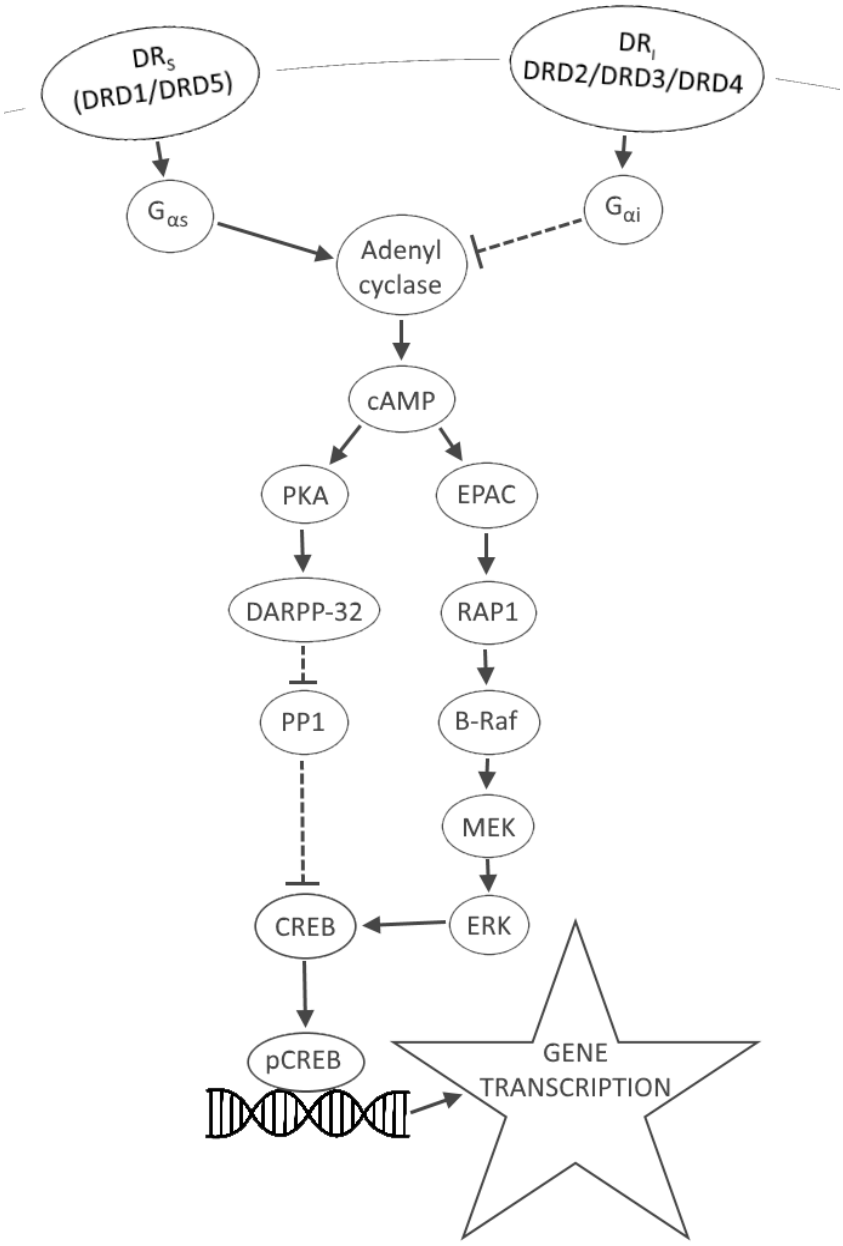

Figure 2: Dopamine receptors act on adenyl cyclase to stimulate or inhibit CREB-mediated gene transcription. The D1-like receptors (DRD1, DRD5) stimulate adenyl cyclase and downstream cascades which lead to phosphorylation of CREB and resulting gene transcription. Conversely, the D2-like receptors (DRD2, DRD3, DRD4) inhibit adenyl cyclase and prevent these downstream effects. DR_S, stimulatory dopamine receptors; DR_I, inhibitory dopamine receptors; $G\alpha_s$: stimulatory G-protein alpha subunit; $G\alpha_i$: inhibitory G-protein alpha subunit; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; EPAC: exchange protein activated by cAMP; DARPP-32: dopamine- and cAMP-regulated neuronal phosphoprotein; RAP1: Ras-related protein 1; B-Raf, serine/threonine-protein kinase B-Raf; PP1: protein phosphatase 1; MEKs: Mitogen-activated protein kinases; ERK: extracellular-signal-regulated kinase; CREB: cAMP response element binding protein; pCREB: phosphorylated CREB. Arrows denote activation, dotted lines denote inhibition.

There is some disagreement in the existing literature as to how catecholaminergic tyrosine hydroxylase expression is affected by chronic stress. However, in a review of existing literature, Sabban and Kvetňanský (2001) concluded that *Th* mRNA and immunoreactivity levels are increased in the ventral tegmental area (a region containing a relatively high concentration of dopaminergic neurons) as a result of chronic stress, and this is consistent with what is known of stress-related activation of the hypothalamic-pituitary-adrenal (HPA) axis. HPA activation leads to increased production of glucocorticoids, and in binding to their receptors, they increase phosphorylation and activation of the cAMP response element binding protein (CREB), a transcription factor for which a response element is found alongside the NGFI-B response element (NBRE) in the promoter region of *Th* (Ghee, Baker, Miller, & Ziff, 1998). Therefore, while TH is regulated at the transcriptional level by NURR1, it has also been demonstrated that *Th* transcription is also inhibited by the downstream effects of increased catecholamine concentration (Meiser, Weindl, & Hiller, 2013). Consequently, dopamine itself also contributes to negative feedback of catecholamine synthesis through decreasing CREB-mediated synthesis of TH.

AADC, on the other hand, is regulated primarily by DA receptor-mediated phosphorylation. Treatment with dopaminergic antagonists such as SCH23390 or haloperidol has been shown to result in increased AADC activity (Hadjiconstantinou & Neff, 2008; Zhu, Juorio, Paterson, & Boulton, 1993). Furthermore, okadaic acid, a protein phosphatase I (PP1) inhibitor, also results in increased AADC activity (Hadjiconstantinou & Neff, 2008). This suggests that antagonism of the D_1-like

excitatory receptors leads to decreased inactivation of PP1, and as a result, greater dephosphorylation and inactivation of AADC. In this way, AADC is also regulated by dopaminergic feedback.

In addition to enzymes which regulate dopamine synthesis and further metabolism, dopamine's concentration is also regulated, specifically in the synapse, by transport proteins in the cell membrane of the presynaptic axon. The vesicular monoamine transporter 2 (VMAT2) is an integral membrane protein that, as the name suggests, facilitates transport of monoamines into vesicles. According to a literature review by Kvetňanský, Lu, and Ziegler (2013), only VMAT2, not VMAT1, is expressed in neurons, and VMAT2 has a tendency to colocalize with TH. It has been previously demonstrated that expression of VMAT2 is downregulated in response to chronic stress in the nucleus accumbens (Zucker, Weizman, & Rehavi, 2005), and since vesicular transport is necessary for neurotransmitter release, this further contributes to decreased extraneuronal dopamine levels. There is also general agreement about the effects of chronic stress on expression of *Dat1*. In various neural structures including the nucleus accumbens (Scheggi *et al.*, 2002), caudate nucleus, and putamen (Isovich, Mijnster, Flügge, & Fuchs, 2000), increases seen in extraneuronal dopamine levels are generally attributed to decreased expression of DAT on the presynaptic neuron. Once dopamine is removed from the synapse, it is broken down by a number of catecholamine degradation enzymes, including monoamine oxidase (MAO). Kvetňanský *et al.* (1984) demonstrated that MAO activity is decreased in the hypothalami of animals subjected to repeated

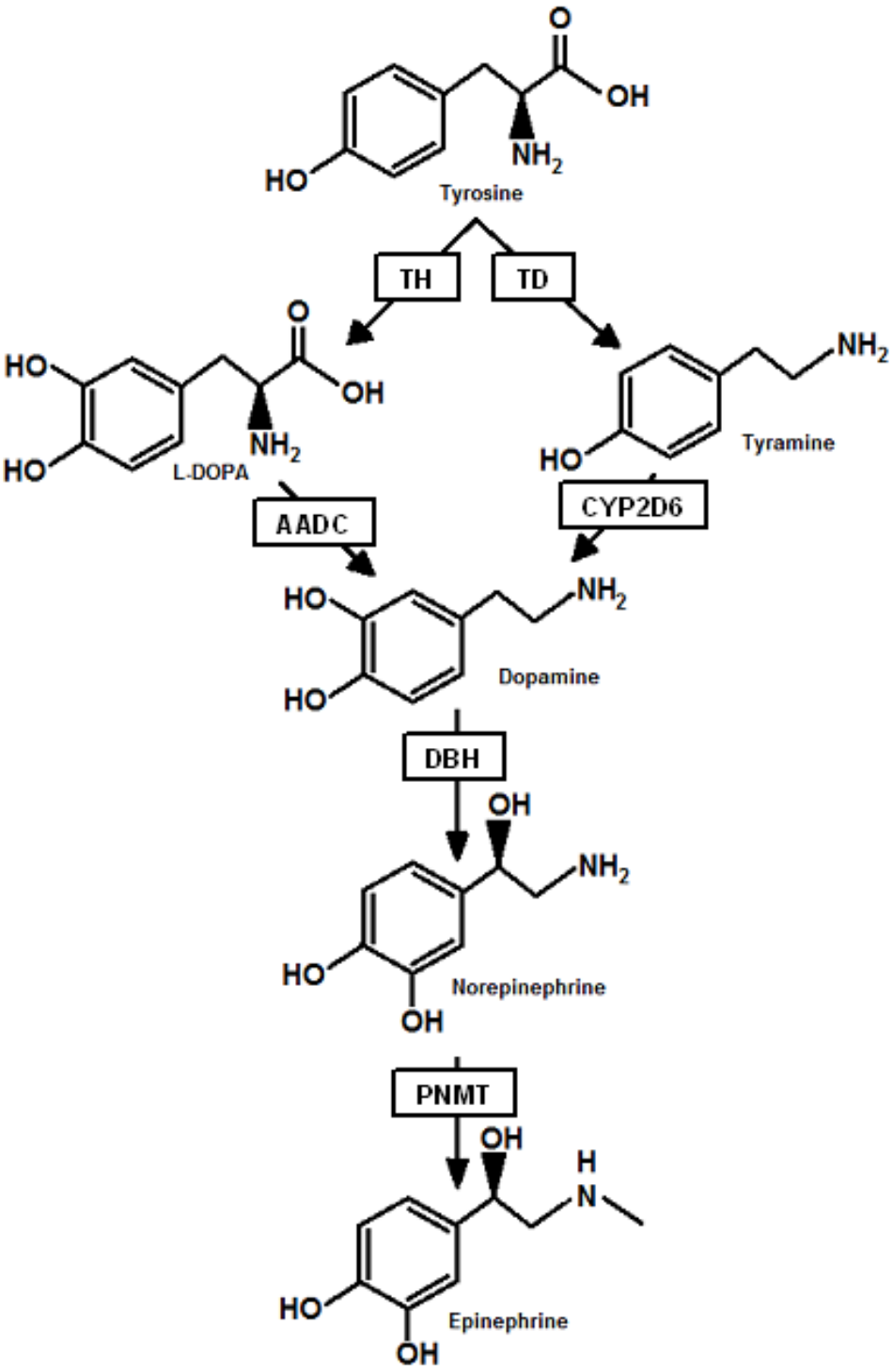

Figure 3: The catecholaminergic synthesis pathway. TH: tyrosine hydroxylase; TD: tyrosine decarboxylase; AADC: aromatic L-amino-acid decarboxylase; CYP2D6: cytochrome P450 2D6; DBH: dopamine β-hydroxylase; PNMT: phenylethanolamine N-methyltransferase.

stress, which downregulates the process of catecholamine degradation.

To summarize, there are five subtypes of dopamine receptor, which are differentially expressed across various regions of the brain. The D_1-like excitatory receptors stimulate the cAMP pathway and upregulate transcription of CREB target genes, while the D_2-like inhibitory receptors inhibit cAMP. The abundance of dopamine itself is regulated by a number of enzymes—those upstream which facilitate conversion of tyrosine to dopamine, and those downstream which convert dopamine into norepinephrine and epinephrine. Synaptic concentration of dopamine is regulated by transporters which facilitate the reuptake of excess dopamine into the presynaptic neuron, as well as enzymes which degrade the dopamine once it has been reabsorbed. As this network of genes demonstrates, dopaminergic signaling is highly regulated.

NURR1

NURR1 (also known as NR4A2) is a member of the nuclear receptor family of proteins which has been implicated in transcription of genes necessary for dopaminergic regulation. Nuclear receptors are a class of proteins which bind to hormone response elements (HREs) in promoter regions of DNA to activate transcription of genes coupled to the specific receptor's RE. Once bound, these receptors initiate transcription through the recruitment of additional complex substituents, such as coactivators and/or additional transcription factors (Wärnmark, Treuter, Wright, & Gustafsson, 2003). Khorasanizadeh and Rastinejad (2001) characterized these proteins as sharing a structure consisting of several features: a degenerate amino terminus, hinge region, and conserved DNA-binding (DBD) and receptor-specific ligand binding (LBD) domains. However, structural studies

have indicated the lack of a ligand-binding pocket within the native conformation of NURR1, leading to its classification as an orphan receptor (Wang *et al.*, 2003), as illustrated in Fig. 4.

NURR1 is first expressed in the midbrain at day 10.5 of embryogenesis, but its presence continues into maturation and adulthood (Torii*,* Kawarai, Nakamura, & Kawakami, 1999; Sakurada, Ohshima-Sakurada, Palmer, and Gage, 1999). It is believed to be transcriptionally activated by the NF-κB and the cyclic AMP response element binding protein (CREB) factors, based on binding assays involving the *Nurr1* promoter region (McEvoy *et al.*, 2002; Kovalovsky *et al.*, 2002), and according to Liu, Serova, Kvetňanský, and Sabban (2008), immobilization stress has been demonstrated to induce transcription of *Nurr1* in the adrenal medulla. There are multiple 5'-ATTTA-3' sequences in the 3' untranslated region of the gene, a sequence which is known to cause mRNA instability and is suggestive of an immediate early gene, or IEG (Torii *et al., 1999*).

NURR1 is a member of the Nur77 receptor family, alongside Nur77 and NOR-1, the three show a large amount of sequence homology, sharing the NBRE consensus sequence consisting of the sequence 5'-AAAGGTCA-3' (Sacchetti, Mitchell, Granneman, & Bannon, 2001; T. Kim *et al*., 2013). Although an orphan receptor, there have been cases where RXR has been demonstrated to dimerize with NURR1 in a mechanism which is believed to promote increased expression of target genes (T. Kim *et al.,* 2013), and at least one study has demonstrated dysfunctional dopamine signaling and locomotion in an RXR knockout mouse model (Kręzel *et al.*, 1998). However, RXR is not necessary for expression of all NURR1 targets (Sacchetti *et al*., 2001).

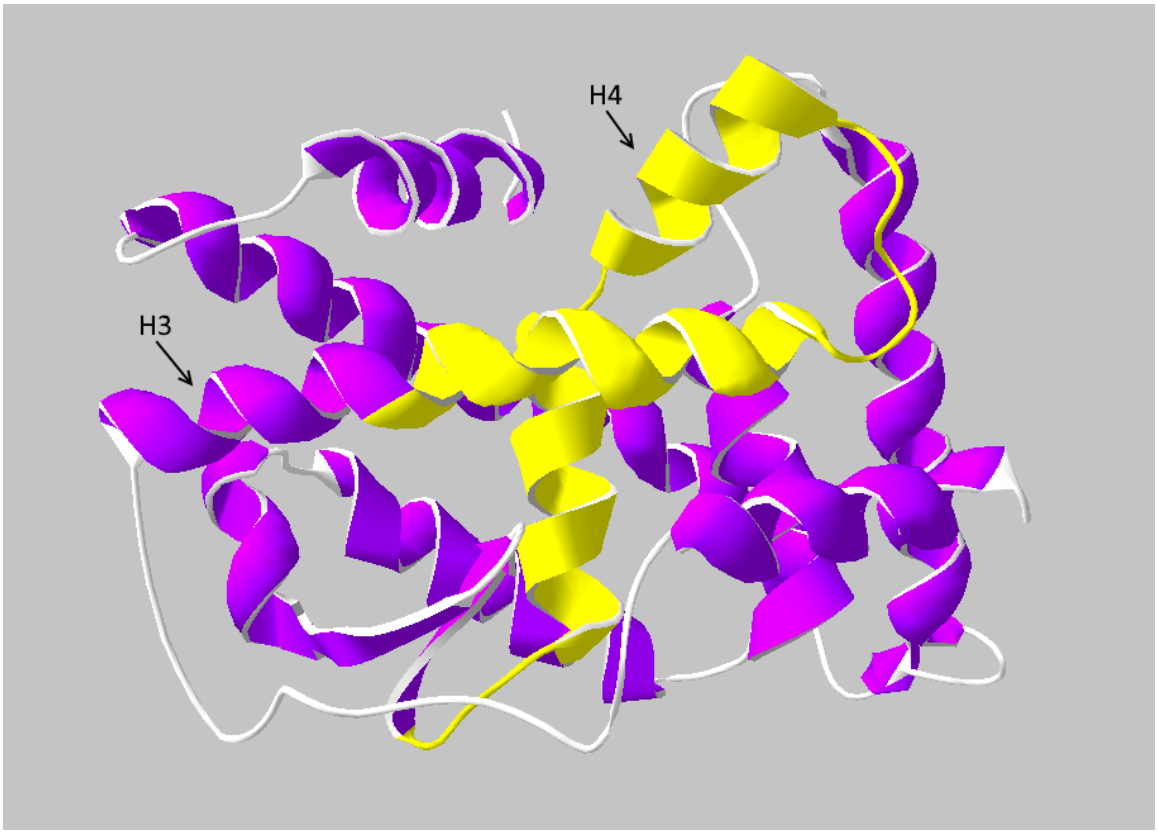

Figure 4: Crystal structure of NURR1 (Helix 1, DNA binding domain not pictured). Notice that the traditional position of the ligand binding domain (between helices 3 and 4, in yellow) is blocked due to close proximity of hydrophobic residues. Image generated from PBD 1OVL, residues 363-598, using DeepView/SPDBV (v. 4.1.0, Swiss Institute of Bioinformatics, Lausanne, Switzerland.)

There is a lack of differentiation of dopaminergic neurons in *Nurr1*-null mice (Kadkhodaei *et al*., 2009), and overexpression of the gene induces mesenchymal stem cell differentiation into dopaminergic neurons (Park *et al.*, 2012), suggesting a major role for the gene in neuronal development. However, it is also implicated as a major regulatory component of mature dopaminergic neurons, as it has been demonstrated to act as a transcriptional activator of proteins crucial for dopaminergic signaling, such as tyrosine hydroxylase (Sakurada *et al*. 1999; T. Kim *et al.*, 2013) and the dopamine transporter (Sacchetti *et al.,* 2001; Bannon *et al.*, 2002). Because of this role, NURR1 has garnered interest as a target for therapeutic interventions aimed at dopamine-related

psychiatric disorders. Polymorphisms and mutations of the gene have been linked to Parkinson's disease (Carmine *et al.*, 2003), bipolar disorder, and schizophrenia (Buervenich *et al.*, 2000), and tissue analyses have found downregulation of NURR1 in both peripheral neurocytes and dopaminergic neurons of PD patients (Kadkhodaei *et al*., 2009). Furthermore, it has been demonstrated that a deficiency in NURR1 leads to a decrease in dopamine levels, which may be the mechanism by which it leads to Parkinson's (Park *et al.,* 2012). A *Nurr1*-heterozygous mouse model showed hyperactivity which was reduced by administration of haloperidol, a DRD2 antagonist, as well as an increase in immobility during the Forced Swim Test (indicating depression), consistent with symptoms of schizophrenia (Rojas, Joodmardi, Hong, Perlmann, & Ögren, 2007).

Dopamine Transporter (Dat1)

The dopamine transporter (also known as SLC6A3) is a protein found in the synaptic membrane that facilitates the reuptake of synaptic dopamine into presynaptic terminals (Sacchetti *et al.,* 2001; Felten, Montag, Markett, Walter, & Reuter, 2011). It has been demonstrated that *Dat1* is induced by NURR1 independently, without the necessity of RXR binding. Furthermore, it has been demonstrated that binding to the NBRE is not necessary for NURR1 activation of *Dat1* transcription, a phenomenon which has not been otherwise observed in the Nur77 family; but which has been demonstrated in the interactions of other nuclear receptors (Sacchetti *et al*., 2001).

There is a variable number of tandem repeats (VNTR) sequence present in the *Dat1* gene, and this polymorphism alters transporter density, resulting in a downstream

effect on synaptic dopamine concentration (Felten *et al.,* 2011). However, the polymorphism showed no significant effect on results of the Affective Neuroscience Personality Scales (Felten *et al.,* 2011), the Schedule for Affective Disorders and Schizophrenia-Lifetime Version, and the Structured Clinical Interview for DSM-IV—Patient Version (Frisch *et al.*, 1999). Furthermore, meta-analyses of existing literature showed that the polymorphism showed no association with schizophrenia (Gamma, Faraone, Glatt, Yeh, & Tsuang, 2005; Joober *et al.*, 2000). Nevertheless, it is believed that DAT pathology may play a role in the same disorders as NURR1, along with disorders involving attention deficits and addiction (Joober *et al.,* 2000). Rowe *et al.* (1998) presented data to suggest that this VNTR polymorphism may play a role in the pathology of "internalizing disorders:" generalized anxiety, social anxiety, phobias, obsessive-compulsive disorder, and Tourette's syndrome.

Tyrosine Hydroxylase (TH)

As mentioned previously, tyrosine hydroxylase is the rate-limiting enzyme in catecholamine synthesis (Thibaut *et al.*, 1997), which converts the amino acid tyrosine into L-DOPA. In rodents, it has been demonstrated that TH is necessary for dopaminergic functioning; however, in humans, while it has been confirmed that the NBRE is present in the *Th* promoter region (Jin *et al.*, 2006), it is not strictly necessary, as the CYP2D6 pathway presents an alternative method of dopamine synthesis, and it has been demonstrated that siRNA downregulation of human NURR1 transfected into an animal model did not affect TH expression (Jin *et al.*, 2006). Further investigation showed that the sequence homology between human and murine *Th* promoter regions is only 46.4%

(Jin *et al.*, 2006). Despite its biological redundancy in humans, it is included in this study because of its known significance in rodent models.

Despite this redundancy, however, mutations in the human *Th* gene can still produce a phenotype consistent with certain psychiatric disorders. A perfect ten tetranucleotide repeat located in the first intron of the gene shows a significant correlation with schizophrenia, whereas a 1 base-pair deletion within this sequence is considered wild-type (Thibaut *et al*., 1997). A postmortem analysis of depressive patients and suicide victims demonstrated an upregulation in TH transcription in the locus coeruleus, a region of the brainstem, which the authors believed to be an adaptive mechanism based on low norepinephrine levels (Zhu *et al.*, 1999). This suggests that conversely, a functional mutation in TH may also cause depressive symptoms, as without it, neurons which lack the necessary enzymes for the secondary dopamine synthesis pathway may present with low norepinephrine levels. Polymorphisms in CYP2D6 have been linked to tardive dyskinesia and other movement disorders in response to second-generation antipsychotic medications, suggesting that it plays an equally vital role in dopamine synthesis (Arranz, Blanco, & Samperiz, 2016). Additionally, another study of TH and symptomology found a significant link between certain allelic variations of the gene and occurrence of bipolar disorder (Serretti *et al.*, 1998).

Despite the evidence that an otherwise healthy individual can overcome abnormally low TH levels by alternate dopamine synthesis pathways, if this abnormality is compounded with abnormalities in sister pathways, it can cause a number of psychiatric symptoms. Therefore, as with DAT, there is a possibility that low TH levels

can be responsible for NURR1-related symptoms in individuals with an irregularity in CYP2D6 or its synthesis.

Epinephrine and the β-Adrenergic Receptors

As mentioned previously, epinephrine/adrenaline is the final endpoint of the catecholaminergic synthesis pathway, and is the main neurotransmitter implicated in the physiological manifestations of the "fight-or-flight" response. The adrenergic receptors are grouped into two classes of G-protein coupled receptors by their effects on systemic circulation, α (vasoconstrictive) and β (vasodilating), and of the latter, there are three subtypes (Waarde, Vaalburg, Doze, Bosker, & Elsinga, 2004). All three β-receptors are coupled to the excitatory $G\alpha_s$ protein (Gurdal, Friedman, & Johnson, 1995; Sidhu, 1998), thereby leading to activation of the same adenyl cyclase pathway as the excitatory dopamine receptors and culminating in increased phosphorylation of CREB.

A study by Patki, Atrooz, Alkadhi, Solanki, & Salim (2015) showed increased hippocampal (as well as prefrontal cortical and amygdalar) epinephrine in response to a several-day social defeat paradigm. H. Kim *et al.* (2013) also demonstrated that a control group of animals exposed to chronic cold stress showed a significant upregulation in transcription of phenylethanolamine N-methyltransferase (PNMT), as well as a significant decrease in dopamine and a significant increase in adrenaline levels in the hypothalamus and hippocampus.

II. HYPOTHESIS AND AIMS

Based on what has been previously discovered about the roles dopamine plays in learning, memory, and emotion, we hypothesize that transcription of genes which encode proteins that contribute to the synthesis and signal transduction of dopamine is higher in the hippocampi of animals which display higher levels of spatial and emotional memory. In particular, we focused on the following areas and aims:

- Aim 1: To assess the relationship between dopamine receptor gene expression and behavioral performance following chronic stress exposure in mice.
- Aim 2: To test whether expression of genes that facilitate dopaminergic signaling in the hippocampus (*Nurr 1* and *Th*) is positively associated with behavioral performance.
- Aim 3: To test whether expression of a gene that inhibits dopaminergic signaling in the hippocampus (*Dat1*) is negatively associated with behavioral performance.

In regards to Aim 1, although the D_1-like excitatory and D_2-like inhibitory dopamine receptors serve opposite roles in transmission of the dopaminergic signal, we anticipate an overall increase in mRNA expression across all subtypes in higher-performing animals (in line with Aim 2), rather than a bidirectional change dependent on receptor subtype. In Aim 2, *Nurr1* is a nuclear receptor which facilitates transcription of dopamine-regulation genes, and *Th* is necessary for the synthesis of dopamine from tyrosine. In Aim 3, *Dat1* is

responsible for facilitating reuptake of dopamine into the presynaptic neuron, thereby preventing postsynaptic binding. By analyzing a variety of genes involved in different aspects of this system, the goal of the current study is to determine which aspect may be responsible for memory deficits resulting from dopamine dysregulation.

III. MATERIALS AND METHODS

Ethics Statement

All procedures involving live animals were performed consistent with the standards set forth by the National Institutes of Health and the National Research Council's *Guide for the Care and Use of Laboratory Animals*. Studies were approved by the Wright-Patterson Air Force Base Institutional Animal Care and Use Committee.

Responsibilities of the Author

Although the author of the current study performed the subsequently mentioned animal handling procedures as part of her laboratory responsibilities, all animal handling in the current study was performed prior to her membership in the lab, as described in Carhuatanta, Shea, Herman, and Jankord (2014). RNA sequencing analysis was performed by Dr. Seung Ho Jung. The author of the current study designed the hypothesis and research aims, and was provided the expression data derived from RNA sequencing, as well as raw behavioral data, and performed the statistical analysis.

Animals

9 week old male BXD (C57BL/6J and DBA/2J recombinant inbred strain, Jackson Laboratory, Bar Harbor, ME, USA) mice were housed individually and given standard food and water *ad libitum.* Prior to the experimental paradigm, animals were allowed to acclimate to the animal facility for a period of ten days. The facilities were climate controlled under the following parameters: 18-24°C, 30-70% humidity, 12 hour

dark/light cycle. All experimental handling occurred Monday through Friday of each week, during the light cycle.

Chronic Variable Stress

All animals were subjected to a four-week Chronic Variable Stress regimen, during which subjects were exposed to up to two of the following stressors each day (5 days per week): 15 minutes of cold (4°C) exposure, 30 minutes of hypoxia, 1 hour of cage placement on a shaker, novel housing overnight, and 30 minutes of open field exposure. The sequence of stressors was randomized to prevent habituation; however, all animals were subjected to an identical sequence. The handling schedule is summarized in Table 1.

	Monday	Tuesday	Wednesday	Thursday	Friday
1	Open Field Cage Shaking	Hypoxia Overnight Novel Housing	Cold Exposure	Hypoxia	Cold Exposure Cage Shaking
2	Hypoxia Open Field	Cold Exposure Elevated Plus Maze	Morris Water Maze	Cage Shaking Morris Water Maze	Open Field Morris Water Maze
3	Morris Water Maze Hypoxia	Morris Water Maze Open Field	Morris Water Maze Cage Shaking	Morris Water Maze Overnight Novel Housing	Cold Exposure
4	Open Field Fear Conditioning	Cage Shaking Fear Conditioning	Fear Conditioning	Cold Exposure Hypoxia	**Tissue collection**

Table 1: Summary of stressors and behavioral assays over the experimental period.

Elevated Plus Maze

The Elevated Plus Maze is a behavioral assay which serves to quantify an animal's anxiety level (Hogg, 1996). The maze was elevated 1 meter from the floor and consisted of two pairs of 40 cm x 8 cm platforms arranged in a cross: one in which the arms are open to the air, the other enclosed with 29.5 cm tall walls constructed of gray plexiglass. The natural inclination of rodents is for dark, enclosed spaces, and therefore, lack of anxiety is measured by the percent of the total test time (5 minutes) which is spent exploring the open arms. Video of each trial was recorded and duration spent in each arm

was automatically calculated using EthoVision XT 7.0.418 tracking software (Noldus Information Technology, Leesburg, VT, USA).

Morris Water Maze

The Morris Water Maze is a behavioral assay used to examine subjects' spatial memory and learning abilities (Morris, 1984). Briefly, subjects are placed into a pool of opaque water, under the surface of which is a solid platform which when stood upon, enables the majority of the animal's body to remain above water. During a single day, subjects are placed into the pool in varying quadrant positions, and are provided with environmental directional cues (large varying printed symbols positioned above the pool). Various measures of each trial (4 per animal per day) are recorded, including subject position, swim path, speed, and latency to platform. Subjects completed four trials per day, for five days, followed by a one day learning extinction procedure during which the platform was removed from the pool, and on the final day, post-extinction recall was tested. The final two days of the study were performed for parallel studies; the current study examined average latency to platform over 20 trials (4 trials per day across the first five days). All trials were recorded using EthoVision XT.

Fear Conditioning

Fear conditioning, as the name suggests, is utilized as an assessment of emotional learning and memory (Phillips & LeDoux, 1992). Subjects were placed into sound dampening chambers which contained cameras and consisted of two metal (side) and two plexiglass (front and back) walls (Med Associates, Inc., St. Albans, VT, USA). The first day of the three-day assay consisted of four 3 kHz tones at 85 dB, lasting 30 seconds

each, which were immediately followed by a 2 second, 0.75 mA shock delivered through the floor of the chamber. Between each tone/shock pairing, there was a thirty second interval. Day 1 served to establish classical conditioning, pairing the unconditioned freezing response to fear with the pre-shock tone. On day 2, subjects were placed into the conditioning chamber once more, in the absence of both tone and shock, and freezing behavior (both instances and percent time frozen) were observed. On day 3, the metal grid on the floor of the chamber was replaced with white plexiglass, and freezing behavior was observed in response to a tone in the absence of a shock. Instances of freezing and time frozen on day 3 were analyzed as a measure of emotional memory.

Tissue Collection and RNA Sequencing

The day following the conclusion of all behavioral assays, subjects were euthanized via rapid decapitation, and brain tissue was quickly removed and stored at -80C. Whole hippocampi were dissected from each sample, and total RNA was isolated via an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the provided directions. Isolated RNA samples were shipped overnight to the Next Generation Sequencing facility at UCLA. Whole transcriptome RNA sequencing was performed using an HiSeq 2500 instrument (Illumina, San Diego, CA, USA), and multiplexed files were downloaded from the UCLA database, demultiplexed, and merged into one file.

Bioinformatics

The FASTX-Toolkit (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA) was utilized to remove sequencing adapters (FASTQ Clipper) and remove barcodes and noise (FASTQ trimmer). Quality control of files was validated using FastQC

(Babraham Institute, Cambridge, Cambridgeshire, UK). TopHat (v. 2.0.6, Johns Hopkins University, Baltimore, MD, USA) and Bowtie (v. 0.12.8, Johns Hopkins University) were used to align reads to the *Mus musculus* (NCBIM37) genome assembly hosted by the University of California, Santa Cruz Genome Browser. Once aligned, HTSeq (v. 0.5.3p9, European Molecular Biology Laboratory, Heidelberg, Germany) was used to quantify reads, and DESeq (European Molecular Biology Laboratory) was utilized to normalize the quantifications and discard genes which were not displayed across all samples. Read values were normalized by dividing the raw read count by a scaling factor which accounted for the assumption that the majority of genes analyzed were not differentially expressed. All bioinformatic analysis was performed by Dr. Seung Ho Jung.

Analysis

Normalized read levels for genes of interest from each subject were extracted from processed data. Statistical analysis and generation of graphical data representations were performed using SigmaPlot (v. 13.0, Systat Software, San Jose, CA, USA). Subjects were sorted and grouped by both mRNA expression and individual task performance, and due to the previously mentioned predictions of directional change, the highest and lowest quadrants were compared via one-tailed t-tests. Correlations across the entire population were measured by the Pearson product-moment correlation coefficient ("Pearson's *r*"). Quantitative Trait Loci (QTL) mapping was performed using GeneNetwork (University of Tennessee, Knoxville, TN, USA), with 1000 permutation tests and 2000 bootstrap tests.

IV. RESULTS

RNA sequencing

The hippocampi of all 25 subjects underwent whole-transcriptome RNA sequencing. All of the genes studied displayed a range of relative expression values, as seen in Table 2. The results are visually summarized as well in Fig. 5.

Categorization of groups by mRNA expression

Subjects were grouped into quartiles (n=6, with the exception of *Drd3*, which had 10 subjects displaying a transcription level of zero) according to mRNA expression level. Task performances were compared between the highest and lowest quartiles, and p-value was determined by one-tailed t-test (Figs. 6-8; Table 3). The resulting data shows a statistically significant difference in latency to platform during the Morris Water Maze (spatial memory), as well as instances of and time spent displaying freezing behavior during fear conditioning (emotional memory), between high and low *Nurr1* mRNA expression groups (Fig. 6; MWM $p = 0.0351$; FC $p = 0.009$).

Categorization of groups by task performance

Subjects were grouped into quartiles according to individual task performance. mRNA expression levels were compared between the highest and lowest quartiles, and p-value was determined by one-tailed t-test (Figs. 9-12; Table 4). While we see a similar trend of higher performance (lower latency to platform in MWM, higher time in open arms in EPM, and both lower freezing instances and total time frozen in FC) associated

with higher gene expression, no statistically significant difference was observed. Likewise, no statistically significant change was observed for any task or any other gene studied.

Nurr1 correlations across entire population

Given that initial analysis demonstrated a significant change in task performances across *Nurr1* expression groups, the correlations between performance and expression variables were examined across the entire subject population. While not meeting the threshold for statistical significance, weak negative correlations (as measured by Pearson's *r*) were demonstrated for both latency to platform (MWM) and percent time frozen (FC), as shown in Figs. 13 and 14. A Pearson's *r* value between |0.3| and |0.5| indicates a weak correlation between variables, whereas a value less than |0.3| indicates a negligible correlation, while a positive value represents a positive correlation and negative values represent negative correlations. Given that the Pearson's *r* values for MWM latency to platform and FC total time frozen are solidly in the “weak” range, while not statistically significant, these results are encouraging, and warrant further investigation.

Quantitative Trait Loci mapping

Quantitative traits are polygenic; in other words, there are multiple genes, or regions of genes, which influence the phenotype. To explain simply, a series of associations between genomic regions and phenotype are aggregated to determine which regions are associated with the trait of interest. In the current study, the goal of QTL mapping was to determine what areas of the genome were predictive of hippocampal

Nurr1 mRNA expression. At each region of the subjects' chromosomes, the variant corresponded randomly to either the C57 or DBA strains due to the randomness of genetic recombination, and the higher the peak for a given region of the genome, the higher association there was between a subject's variant at that position and their normalized transcription of *Nurr1.* While no peaks were observed above the likelihood ratio statistic (LRS) significance threshold of 18.21, chromosomes 4, 6, and 11 demonstrated peaks above 11.37, the suggestive threshold (Fig. 15). Closer analysis identified 20 loci across these three chromosomes which may contribute to variation in expression (Table 5). The chromosomal regions which demonstrate peaks associated with *Nurr1* expression, especially on chromosome 11, while not definitively demonstrated to be the cause of the demonstrated behavioral trends, are nevertheless predictive of performance on memory-related tasks. By narrowing down the predictive regions, this paves the way for identification of causative genes and more thorough pathway mapping.

Subject	*Nurr1*	*Dat1*	*TH*	*Drd1*	*Drd2*	*Drd3*	*Drd4*	*Drd5*
354	1516.313	2.777	63.874	569.312	570.700	2.777	4.166	56.931
356	1151.404	2.429	54.655	329.146	344.935	1.215	6.073	125.100
357	1137.651	5.084	49.574	156.348	184.312	2.542	7.627	163.974
360	957.897	10.083	44.366	395.259	316.610	0.000	5.042	110.914
361	1080.989	5.572	18.109	125.372	87.761	4.179	4.179	101.691
362	1339.067	4.894	45.027	139.976	125.293	0.000	6.852	145.849
363	1743.543	3.629	45.358	124.280	146.051	3.629	4.536	117.022
364	470.261	2.995	68.892	530.167	580.089	0.998	8.986	100.842
366	936.489	7.084	63.755	434.950	389.613	0.000	8.501	211.100
368	2182.088	6.033	65.362	474.629	428.373	2.011	13.072	86.479
369	1428.469	7.827	80.229	165.350	184.918	0.000	9.784	196.659
370	1425.458	7.949	173.104	192.534	269.371	1.766	8.832	213.730
371	1667.247	2.267	56.671	1050.672	1204.815	0.000	4.534	37.403
372	991.078	13.530	125.153	121.771	203.797	2.537	10.148	182.656
373	976.615	2.491	72.250	941.736	1005.681	2.491	5.813	45.675
374	722.486	7.713	59.993	110.558	220.260	0.000	5.999	167.980
375	1136.285	6.213	146.016	108.735	112.619	0.777	8.543	219.801
376	878.220	0.930	27.910	104.196	199.088	2.791	6.512	236.301
377	1451.676	5.925	61.227	444.391	357.488	1.975	4.938	209.357
378	2212.255	5.675	68.098	328.197	253.478	0.000	7.566	196.729
379	1255.087	1.749	76.967	348.101	334.107	0.000	5.248	195.916
380	537.599	0.874	59.442	1626.783	2061.232	3.497	4.371	53.323
382	1706.646	1.765	67.948	452.694	327.387	0.000	6.177	112.070
383	1357.424	0.889	64.893	370.691	274.685	0.889	6.223	145.788
384	2083.525	2.005	58.154	328.872	250.665	0.000	7.019	122.324

Table 2: Expression of target gene mRNA expression across the entire studied population

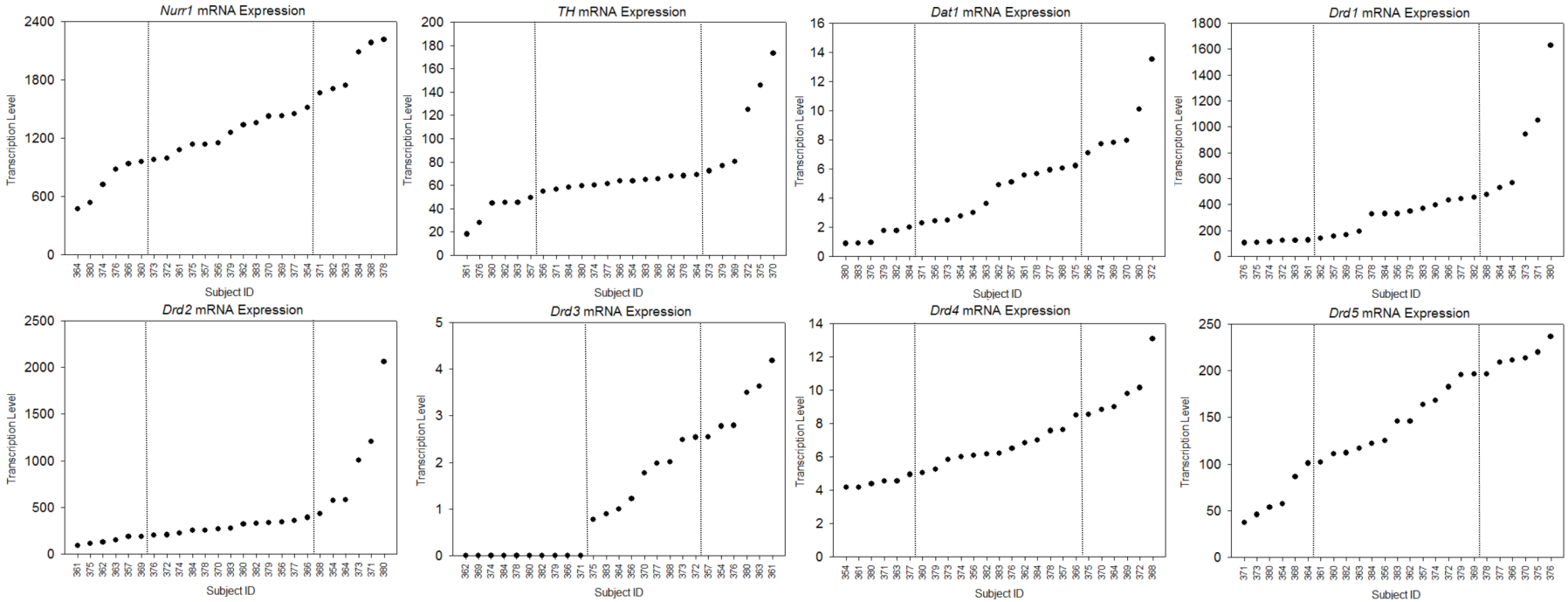

Figure 5: Graphical representation of target gene mRNA expression. Dotted lines represent the divisions between Quartiles 1 and 2, and Quartiles 3 and 4, used for analytical grouping by expression level Range of Quartile 1 for *Drd3* was extended due to identical values of a quantity greater than the standard n=6.

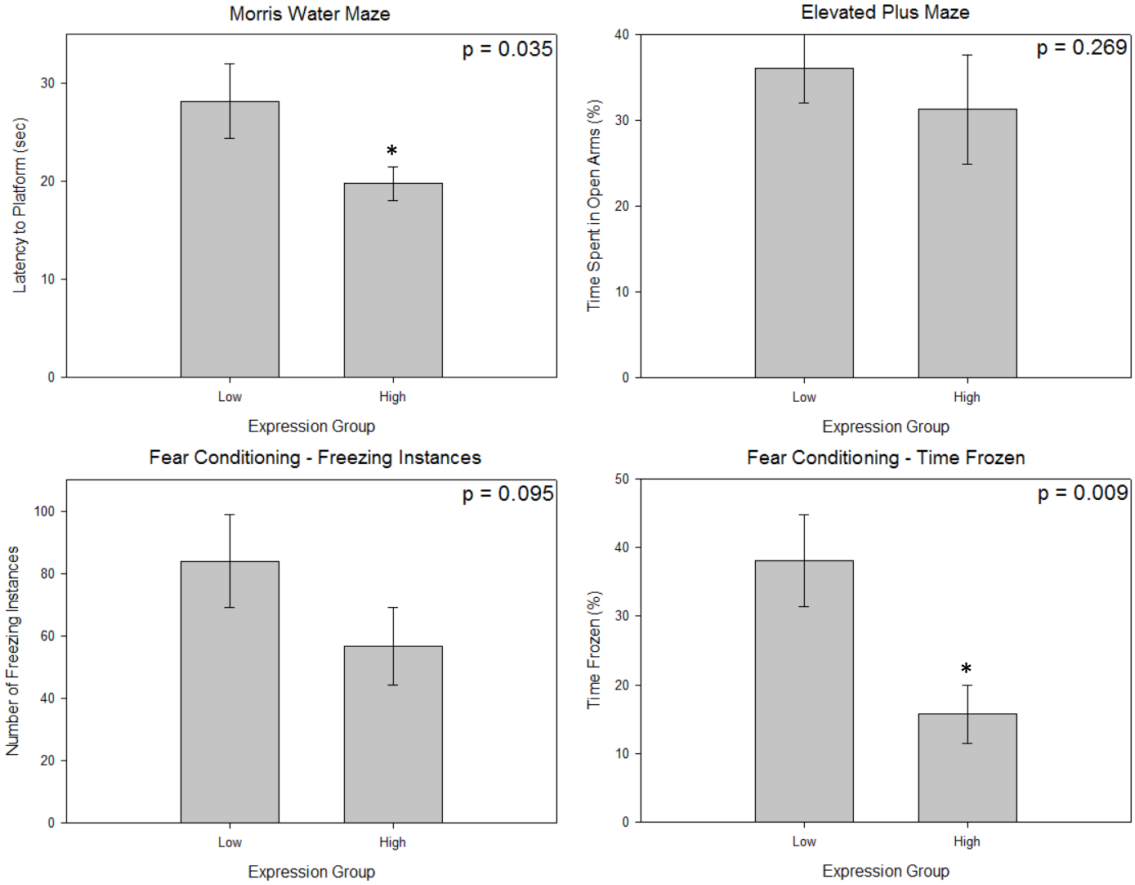

Figure 6: Comparison of task performance between high and low *Nurr1* mRNA expression groups. P-values determined by one-tailed t-test, n=6 for both groups. Notice that Morris Water Maze latency to platform, and time frozen during fear conditioning demonstrated statistical significance between expression groups. Error bars denote standard error. * denotes statistical significance ($p < 0.05$).

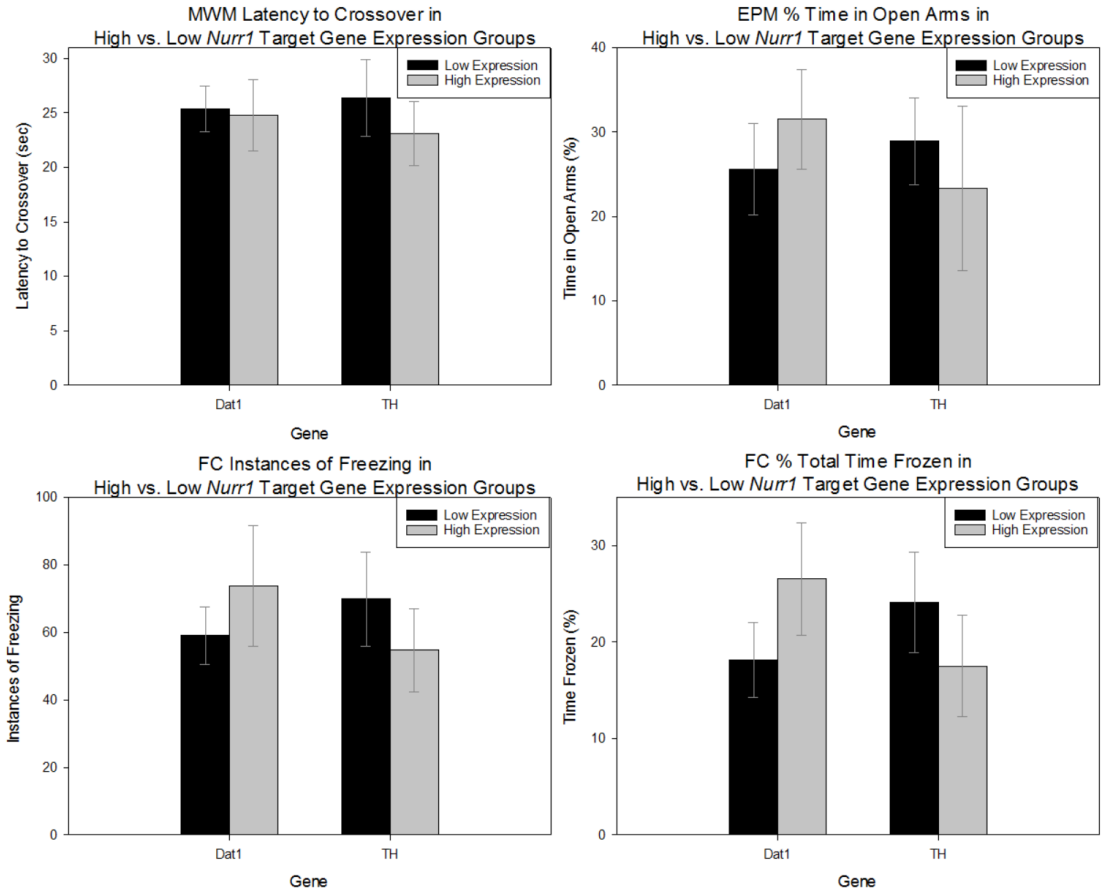

Figure 7: Comparison of task performances between *Nurr1* target gene mRNA expression groups. No statistically significant differences were observed between any expression group pairs. Error bars denote standard error.

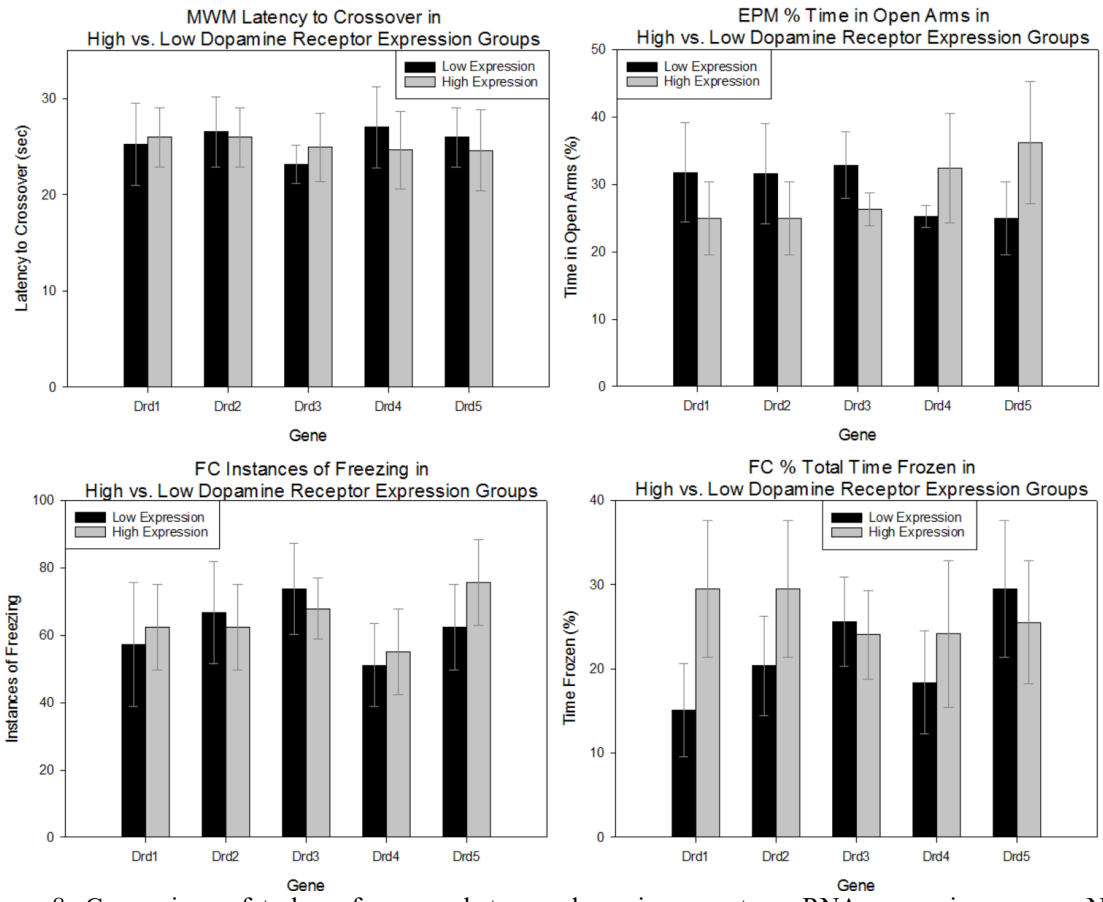

Figure 8: Comparison of task performance between dopamine receptor mRNA expression groups. No statistically significant differences were observed between any expression group pairs. Error bars denote standard error

Gene	MWM	EPM	FC Count	FC Percent
Nurr1	**0.0351***	0.269	0.095	**0.00902***
Dat1	0.224	0.239	0.19	0.0937
TH	0.246	0.312	0.217	0.198
Drd1	0.446	0.236	0.411	0.0877
Drd2	0.454	0.246	0.416	0.193
Drd3	0.319	0.175	0.379	0.426
Drd4	0.346	0.204	0.413	0.299
Drd5	0.399	0.156	0.238	0.727

Table 3: P-values of one-tailed t-tests for subjects grouped by mRNA expression. MWM, average latency to platform in Morris Water Maze; EPM, percent time spent in open arms in Elevated Plus Maze; FC Count, instances of freezing during day three of fear conditioning; FC Percent, percent of total time spent frozen during fear conditioning. * denotes a p-value less than 0.05.

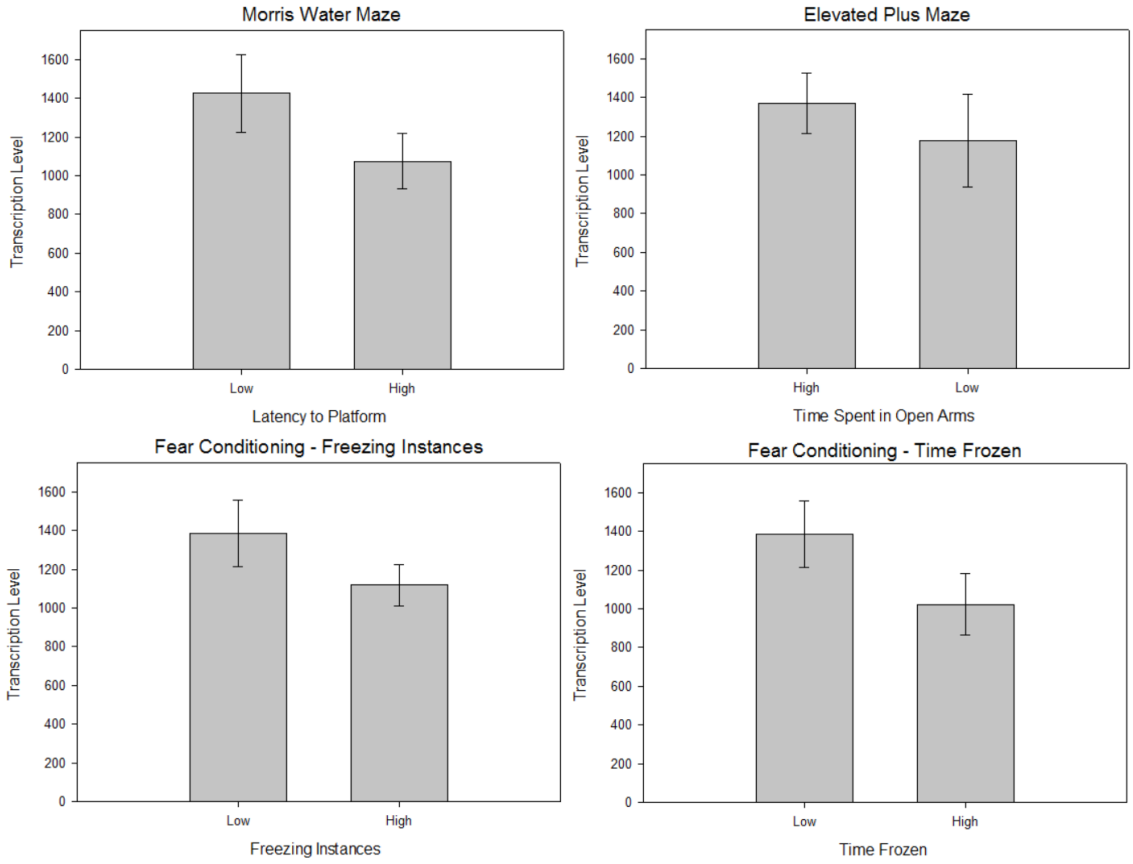

Figure 9: Comparison of *Nurr1* mRNA expression levels between high and low task performances. No statistically significant changes were observed between groups for any of the behavioral assays performed. Error bars denote standard error.

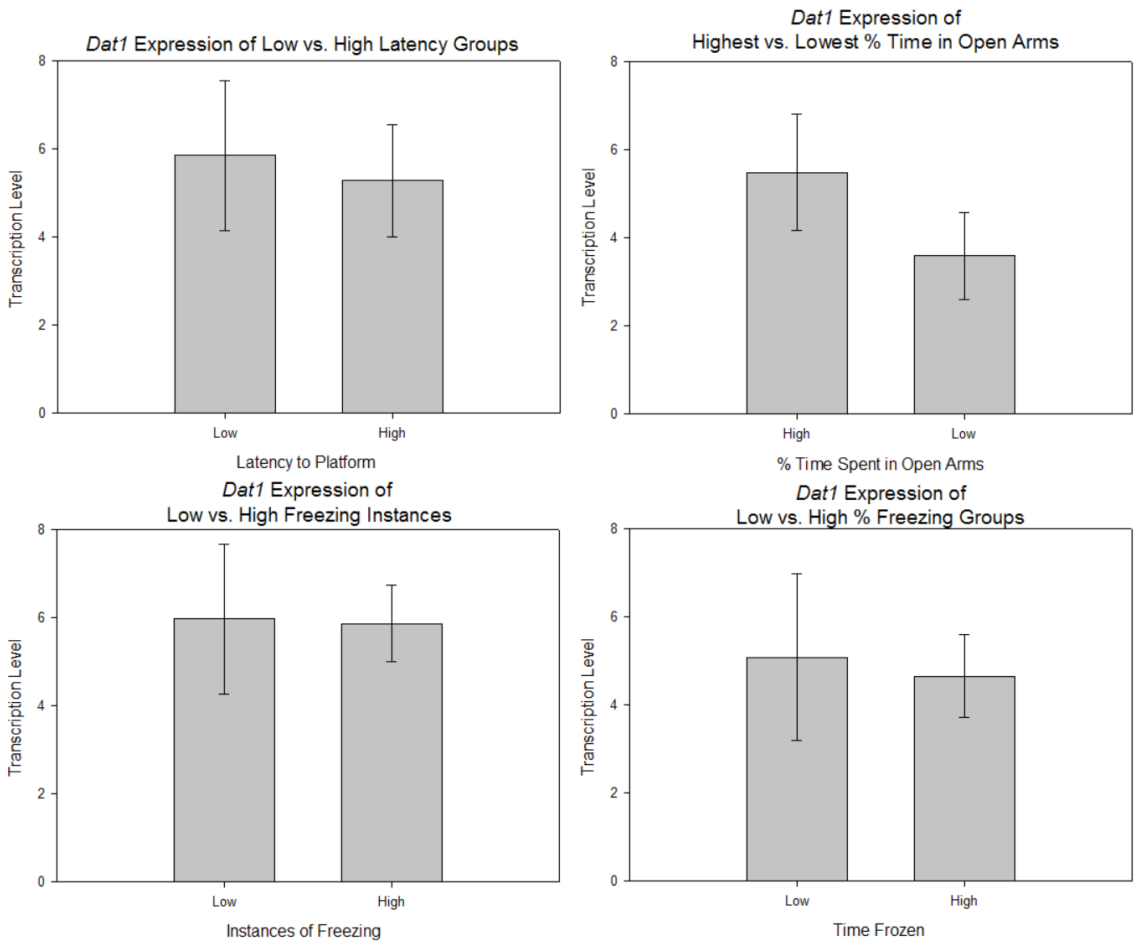

Figure 10: Comparison of *Dat1* mRNA expression levels between high and low task performances. No statistically significant differences were observed between groups for any behavioral assay performed. Error bars denote standard error.

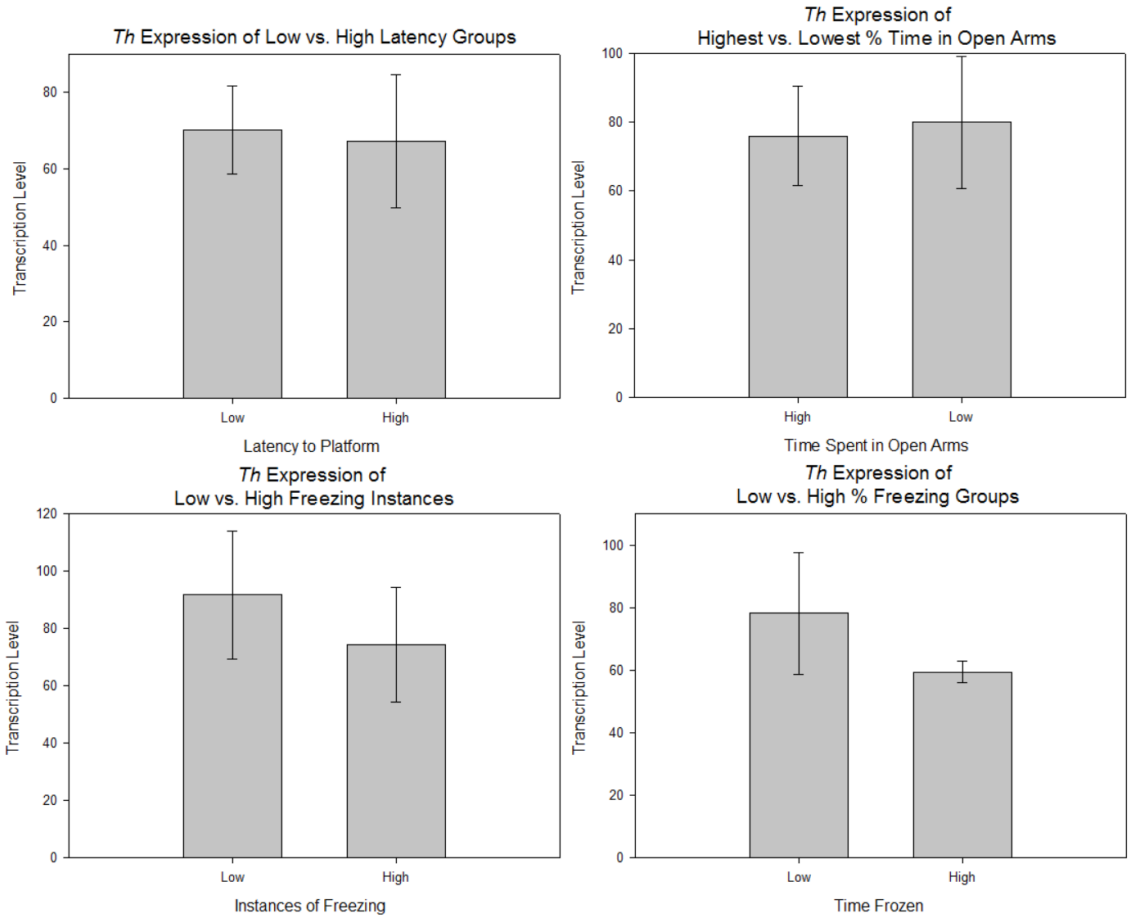

Figure 11: Comparison of *Th* mRNA expression levels between high and low task performances. No statistically significant differences were observed between any performance group pairs. Error bars denote standard error.

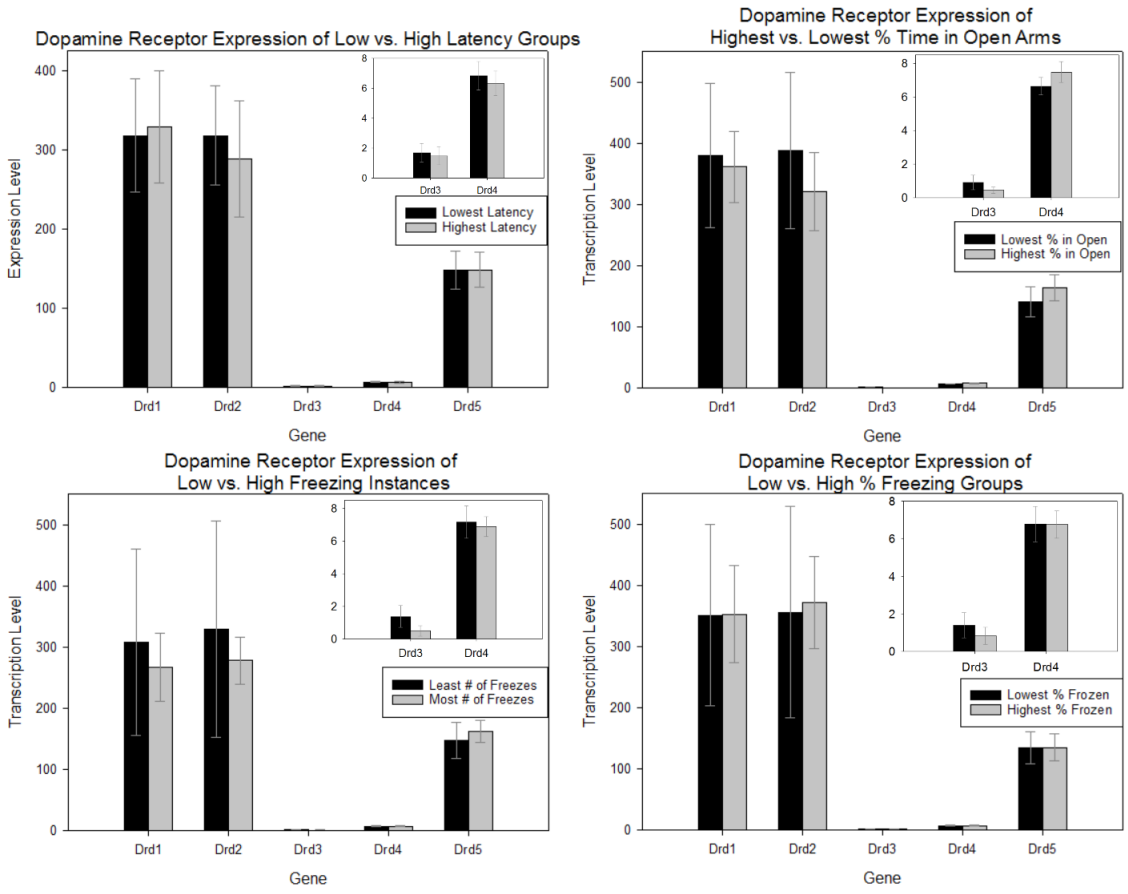

Figure 12: Comparison of dopamine receptor mRNA expression levels between high and low task performances. No statistically significant differences were observed between any performance group pairs. Error bars denote standard error.

Gene	MWM	EPM	FC Count	FC Percent
Nurr1	0.094	0.257	0.0756	0.0866
Dat1	0.396	0.136	0.421	0.364
TH	0.446	0.435	0.183	0.279
Drd1	0.457	0.445	0.497	0.405
Drd2	0.381	0.324	0.467	0.392
Drd3	0.401	0.178	0.25	0.133
Drd4	0.346	0.164	0.496	0.407
Drd5	0.498	0.249	0.496	0.335

Table 4: P-values of one-tailed t-tests for subjects grouped by task performance. MWM, average latency to platform in Morris Water Maze; EPM, percent time spent in open arms in Elevated Plus Maze; FC Count, instances of freezing during day three of fear conditioning; FC Percent, percent of total time spent frozen during fear conditioning.

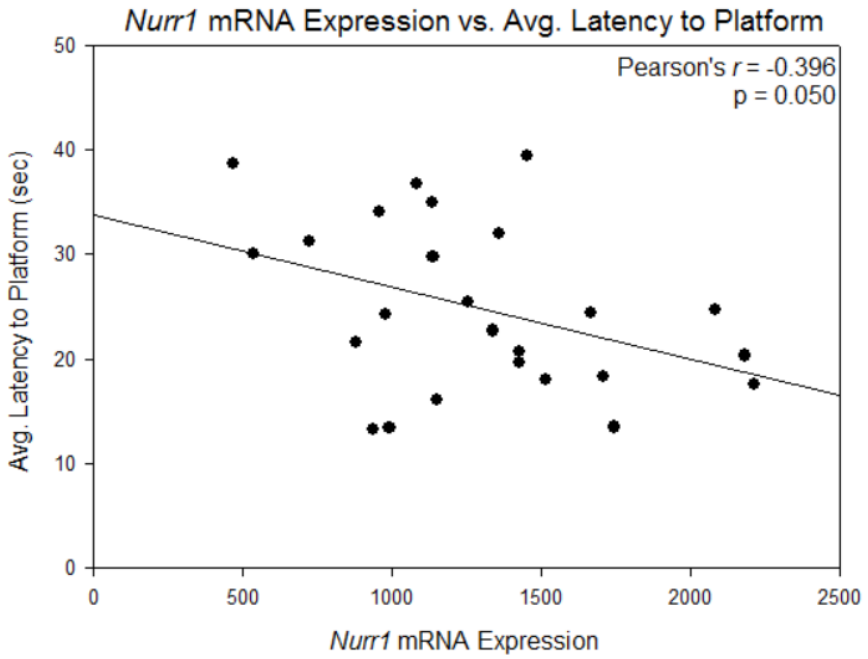

Figure 13: Correlation of *Nurr1* mRNA expression and latency to platform during Morris Water Maze across the entire subject population. The Pearson's *r* value denotes a weak correlation, although the p-value falls outside the range of statistical significance.

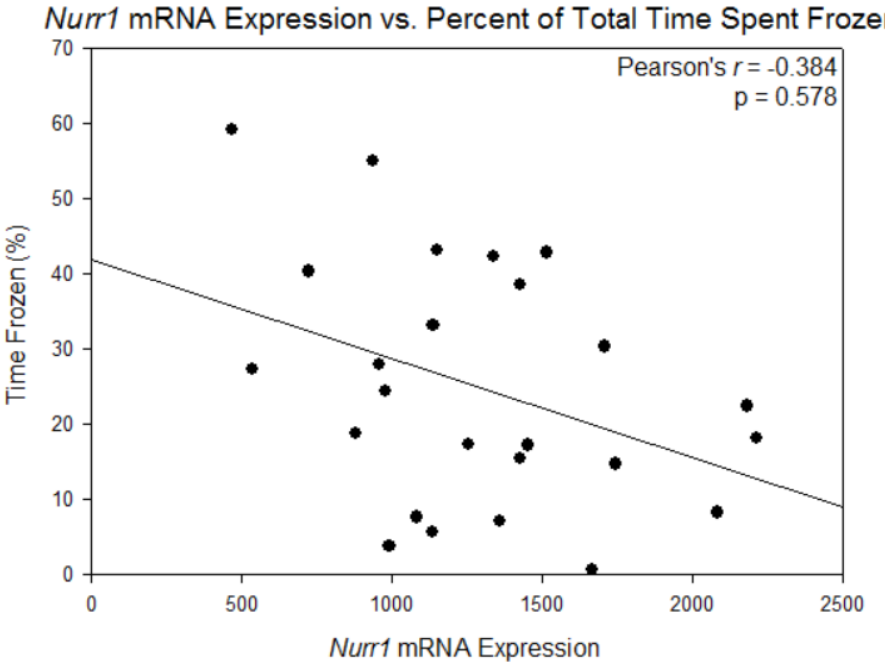

Figure 14: Correlation of *Nurr1* mRNA expression and time spent frozen during day three of fear conditioning. The Pearson's *r* value denotes a weak correlation, although the p-value falls outside the range of statistical significance.

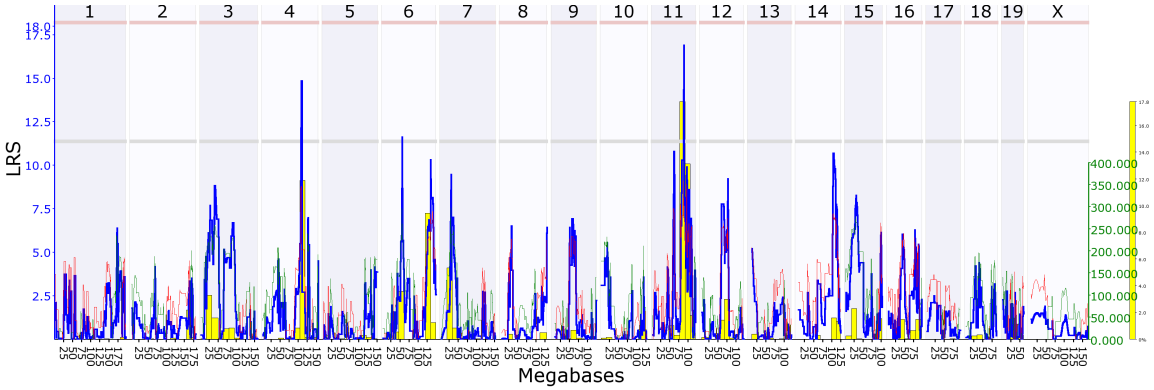

Figure 15: Quantitative Trait Loci mapping of *Nurr1* mRNA expression. Notice that regions within chromosomes 4, 6, and 11 fall above the grey line (the LRS threshold for suggestibility).

Chr	Locus	cM	Mb	LRS	P-value	Additive
4	Unnamed	57.824	108.065	11.942	0.526	-340.554
4	rs3664637	58.166	108.237	12.304	0.474	-338.141
4	rs6226080	58.166	108.383	12.304	0.474	-338.141
4	gnf04.104.549	58.75	108.88	12.304	0.474	-338.141
4	rs3695162	59.335	108.948	14.848	0.203	-345.474
4	mCV24667075	59.335	109.187	14.848	0.203	-345.474
4	D4Mit146	59.622	109.474	14.848	0.203	-345.474
4	rs13477907	59.908	109.542	14.848	0.203	-345.474
4	rs13477910	59.908	110.788	14.848	0.203	-345.474
6	CEL-6_56528034	52.994	56.518	11.614	0.568	295.781
11	rs3659504	69.191	88.523	14.332	0.239	-319.328
11	rs8270514	69.191	88.786	14.332	0.239	-319.328
11	D11Mit41	69.485	88.937	14.332	0.239	-319.328
11	rs6370920	69.771	88.978	14.332	0.239	-319.328
11	gnf11.095.863	69.771	89.106	14.332	0.239	-319.328
11	rs3697441	70.058	89.402	14.332	0.239	-319.328
11	Unnamed	71.058	89.575	16.904	0.078	-358.575
11	rs13481150	71.277	89.613	16.02	0.106	-345.296
11	rs6376709	71.277	89.689	16.02	0.106	-345.296
11	D11Mit179	71.564	89.697	16.02	0.106	-345.296

Table 5: Suggestive loci associated with *Nurr1* mRNA expression (as derived from the QTL mapping illustrated in Fig. 15).

V. DISCUSSION

This study examined the transcription of several genes related to the regulation of dopaminergic signaling. Our results demonstrate that in tests of spatial and emotional memory, there is a positive relationship between transcript abundance of a regulatory element of dopaminergic signaling, *Nurr1*, and task performance (Figs. 6, 13, 14); however, no other studied genes demonstrated such a relationship. Furthermore, they demonstrate that these genes show a range of variation across our subject population, and that in regards to *Nurr1*, there are multiple chromosomal regions which may affect mRNA expression by allelic variation. However, this study also raises the question of whether NURR1 affects memory by dopaminergic regulation, or some other mechanism.

Nurr1

Given that both statistically significant changes between expression groups and weak correlations between transcription and task performance were observed, the present study agrees with previous literature that suggest a major role of *Nurr1* in the studied behaviors. Colón-Cesario *et al.* (2006) found that rats treated with antisense DNA corresponding to *Nurr1* successfully knocked down protein expression and interfered with discrimination of relevance of spatial information during a spatial memory task. In other words, knockdown of *Nurr1* impairs spatial long term memory, thus demonstrating that it plays a vital role . Furthermore, Hawk *et al.* (2012) refer to several previous studies that provide evidence of a role for *Nurr1* in emotional and spatial memory. One such

study demonstrated an increase in *Nurr1* in the CA1 and CA3 regions of the hippocampus *following* hippocampal learning. Another demonstrated that in a heterozygous *Nurr1* null model, mice show impaired emotional memory (measured through a passive avoidance task). The current and previous studies both demonstrate a role for NURR1 in hippocampal memory formation; specifically, in relevance discrimination during memory consolidation.

One detail of special interest is that while a significant change in task performance was observed between groups when subjects were grouped by *Nurr1* expression, the significance did not hold true when subjects were grouped by task performance. This is likely due to the fact that *Nurr1* influences task performance, but is not the only factor to do so. The observation that mRNA expression can weakly predict task performance, but task performance does not predict *Nurr1* mRNA expression, suggests a relationship confounded by the contributions of other signaling cascades. Furthermore, in two of the behavioral measures (latency to platform in the Morris Water Maze and percent time frozen in Fear Conditioning), a weak correlation was demonstrated between *Nurr1* expression and task performance; however, the p-values exceeded the threshold of significance ($p < 0.05$).

Nurr1 targets and dopamine receptors

Despite our initial model which predicted a significant relationship between task performance and expression of the direct targets of NURR1 which were examined in the present study, no significant group differences or correlations were observed. As previously mentioned, vital pathways such as catecholamine synthesis demonstrate the

existence of alternate mechanisms, and furthermore, many genes contain multiple response elements within their promoter regions. In addition to NURR1, both the human and mouse *Th* promoters contain binding elements for HNF-3β, HOXA4, and HOXA5 (Kessler, Yang, Gollomp, Jin, & Iacovitti, 2003). The *Dat1* promoter region likewise contains response elements for CREB and AP-1, along with several potential response elements which were identified (but not experimentally verified) by Bannon *et al.* (2001). While a change was expected, due to the fact that previous literature has demonstrated that mutations or knockdowns of *Nurr1* are associated with dopaminergic dysfunction, transcription of these genes is regulated by multiple pathways. Therefore, this provides a credible explanation for the lack of a relationship between *Nurr1* expression-related task performance, and expression of NURR1 target genes.

Like the target genes of NURR1, no significant correlation was observed between task performances and expression of dopamine receptors. While a relationship was predicted based upon evidence suggesting that synaptic dopamine concentration regulates receptor expression, this is consistent with the lack of correlation in *Th* and *Dat1*, as both these genes play a role in regulation of dopamine's presence in the synapse. Interestingly, while not statistically significant, there are complimentary trends in the Elevated Plus Maze and fear conditioning time frozen data for the dopamine receptors. Low *Drd1* and *Drd2* expression, as well as high *Drd5* expression, are associated with higher time in EPM open arms, indicative of a lower unconditioned anxiety response, and lower time frozen during FC, indicative of a less solid conditioned reaction to an unpleasant stimulus. However, low *Drd3* and high *Drd4* demonstrate a trend toward higher time in

open arms, but lower time spent frozen during fear conditioning. While the similarity in trends between *Drd1* and *Drd5*, as well as *Drd3* and *Drd4* are to be expected as they are grouped together by G-protein response, it is worth noting that *Drd2*, in this instance, demonstrates a trend consistent with the D_1-like receptors.

Interestingly, our observed relative dopamine receptor mRNA levels conflict with previous literature. Meador-Woodruff *et al.* (1994) estimated that hippocampal *Drd2, Drd4*, and *Drd5* levels were roughly equal, while *Drd3* expression was 2- to 5-fold lower, and *Drd1* mRNA levels were 2- to 3-fold lower still. Likewise, Khan *et al*. (1998) stated that *Drd4* mRNA is the most prevalent inhibitory dopamine receptor type in the hippocampus. An examination of mean mRNA level across the entire population for each dopamine receptor subtype in the current study, however, demonstrated that this was not the case. Our results indicated that *Drd2* was the most abundant, followed closely by *Drd1*. *Drd5* was approximately -1.5-fold lower than *Drd1*, and by comparison to the other subtypes, the levels of both *Drd3* and *Drd4* were negligible (Fig. 16). Although the current study provides no non-stressed control for comparison, these studies may suggest that chronic stress changes the relative ratios of hippocampal dopamine receptors.

Animals were restrained for one hour on the morning of the final day, prior to the collection of tissues following euthanasia. Based on previous studies, such as that described by Jankord and Herman (2008), we believe that exposure to this novel stressor led to changes in the mRNA expression profile. Thus, the mRNA expression profiles described in this study represent an interaction between the previous chronic stress and this final acute stressor, which immediately preceded tissue collection. Because tissue

samples were collected approximately one hour after the final stressor, changes in in immediate early genes (IEGs), like *Nurr1*, were likely due, at least in part, to the acute stress response. While this timeframe likely captured changes in IEGs, we would not necessarily expect to see changes in downstream genes, such as *Th* and *Dat1*, which require additional time for transcriptional changes to filter down the signaling cascades.

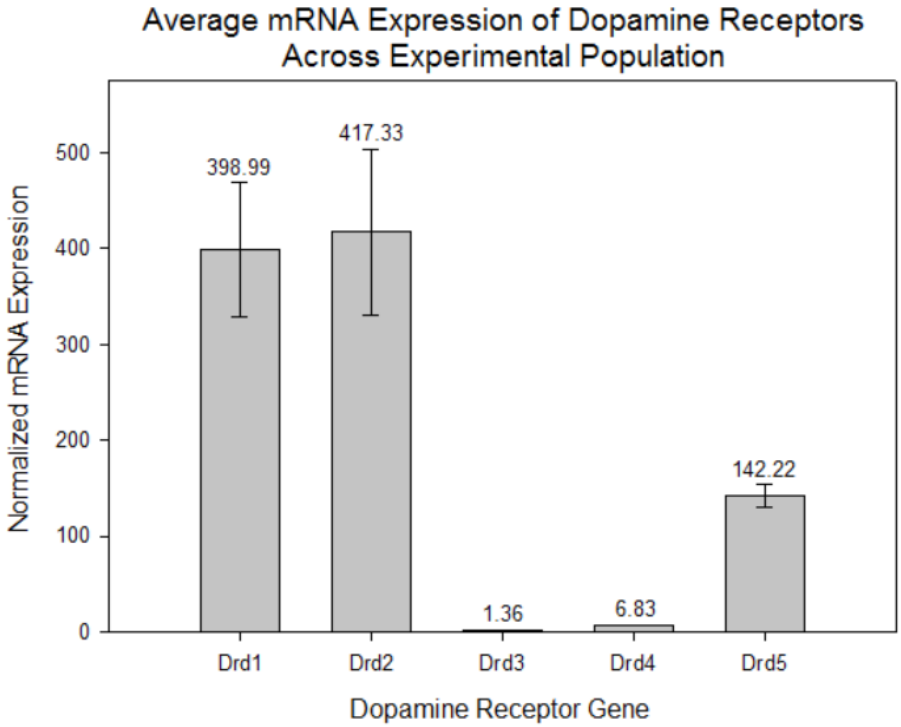

Figure 16: The observed ratios of hippocampal dopamine receptor mRNA levels are not in agreement with previous literature which suggests *Drd4* is the most abundant subtype mRNA. Error bars denote standard error.

Final Thoughts

This study demonstrates that hippocampal expression of *Nurr1* predicts behavioral performance in mice. Our results show that *Nurr1* mRNA transcription levels predict performance during tests of spatial and emotional memory. We did not observe a statistically significant relationship between other dopaminergic genes measured (*Dat1,*

TH, and five subtypes of dopamine receptor) and behavioral performance. Overall, this study is consistent with previous literature that suggests a role for NURR1 in memory, and provides evidence for genetic variability in its regulation. However, it also provides preliminary evidence that NURR1 may also modulate memory-related abilities by some mechanism other than regulation of *Dat1* and *TH* transcription, which may be independent of dopaminergic regulation entirely.

V. REFERENCES

Ahmad, A., Rasheed, N., Banu, N., & Palit, G. (2010). Alterations in monoamine levels and oxidative systems in frontal cortex, striatum, and hippocampus of the rat brain during chronic unpredictable stress. *Stress*, *13*(4), 356-365.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (DSM-5)*. American Psychiatric Pub.

Anders, S., & Huber, W. (2010). Differential expression analysis for sequence count data. *Genome biology*, *11*(10), 1.

Anders, S., Pyl, P. T., & Huber, W. (2014). HTSeq–a Python framework to work with high-throughput sequencing data. *Bioinformatics*, btu638.

Andrews S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Arranz, M. J., Blanco, J. P., & Samperiz, B. A. (2016). Pharmacogenetics of the Efficacy of Antipsychotic Drugs in Schizophrenia. In *Genetic Influences on Response to Drug Treatment for Major Psychiatric Disorders* (pp. 1-20). Springer International Publishing.

Beaulieu, J. M., & Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological reviews*, *63*(1), 182-217.

Buervenich, S., Carmine, A., Arvidsson, M., Xiang, F., Zhang, Z., Sydow, O., ... & Freedman, R. (2000). NURR1 Mutations in cases of schizophrenia and manic

-depressive disorder. *American journal of medical genetics*, *96*(6), 808-813.

Carhuatanta, K. A., Shea, C. J., Herman, J. P., & Jankord, R. (2014). Unique genetic loci identified for emotional behavior in control and chronic stress conditions. *Frontiers in behavioral neuroscience*, *8*.

Ciaranello, R. D., Wooten, G. F., & Axelrod, J. (1976). Regulation of rat adrenal dopamine β-hydroxylase. II. Receptor interaction in the regulation of enzyme synthesis and degradation. *Brain research*, *113*(2), 349-362.

Colón-Cesario, W. I., Martínez-Montemayor, M. M., Morales, S., Félix, J., Cruz, J., Adorno, M., ... & de Ortiz, S. P. (2006). Knockdown of Nurr1 in the rat hippocampus: implications to spatial discrimination learning and memory. *Learning & Memory*, *13*(6), 734-744.

Curtin, S. C., Warner, M., & Hedegaard, H. (2016). Increase in Suicide in the United States, 1999-2014. *NCHS data brief*, (241), 1-8.

Duvernoy, H. M. (2005). *The human hippocampus: functional anatomy, vascularization and serial sections with MRI*. Springer Science & Business Media.

Fahn, S. (2008). The history of dopamine and levodopa in the treatment of Parkinson's disease. *Movement Disorders*, *23*(S3), S497-S508.

Felten, A., Montag, C., Markett, S., Walter, N. T., & Reuter, M. (2011). Genetically determined dopamine availability predicts disposition for depression. *Brain and behavior*, *1*(2), 109-118.

Frisch, A., Postilnick, D., Rockah, R., Michaelovsky, E., Postilnick, S., Birman, E., ... & Schneidman, M. (1999). Association of unipolar major depressive disorder with genes

of the serotonergic and dopaminergic pathways. *Molecular psychiatry*, *4*(4), 389-392.

Gamma, F., Faraone, S. V., Glatt, S. J., Yeh, Y. C., & Tsuang, M. T. (2005). Meta-analysis shows schizophrenia is not associated with the 40-base-pair repeat polymorphism of the dopamine transporter gene. *Schizophrenia research*, *73*(1), 55-58.

Ghee, M., Baker, H., Miller, J. C., & Ziff, E. B. (1998). AP-1, CREB and CBP transcription factors differentially regulate the tyrosine hydroxylase gene. *Molecular brain research*, *55*(1), 101-114.

Gordon, A. (2010). FASTX-Toolkit: FASTQ/A short-reads pre-processing tools. Available online at: http://hannonlab.cshl.edu/fastx_toolkit.

Guex, N. & Peitsch, M.C. (1997). SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis 18*, 2714-2723.

Gurdal, H., Friedman, E., & Johnson, M. D. (1995). Beta-adrenoceptor-G alpha S coupling decreases with age in rat aorta. *Molecular pharmacology*, *47*(4), 772-778.

Hadjiconstantinou, M., & Neff, N. H. (2008). Enhancing Aromatic L-amino Acid Decarboxylase Activity: Implications for L-DOPA Treatment in Parkinson's Disease. *CNS neuroscience & therapeutics*, *14*(4), 340-351.

Hawk, J. D., Bookout, A. L., Poplawski, S. G., Bridi, M., Rao, A. J., Sulewski, M. E., ... & Abel, T. (2012). NR4A nuclear receptors support memory enhancement by histone deacetylase inhibitors. *The Journal of clinical investigation*, *122*(10), 3593-3602.

Hiroi, T., Imaoka, S., & Funae, Y. (1998). Dopamine formation from tyramine by CYP2D6. *Biochemical and biophysical research communications*, *249*(3), 838-843.

Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an

animal model of anxiety. *Pharmacology Biochemistry and Behavior*, *54*(1), 21-30.

Isovich, E., Mijnster, M. J., Flügge, G., & Fuchs, E. (2000). Chronic psychosocial stress reduces the density of dopamine transporters. *European Journal of Neuroscience*, *12*(3), 1071-1078.

Jankord, R., & Herman, J. P. (2008). Limbic regulation of Hypothalamo-Pituitary-Adrenocortical function during acute and chronic stress. *Annals of the New York Academy of Sciences*, *1148*(1), 64-73.

Jin, H., Romano, G., Marshall, C., Donaldson, A. E., Suon, S., & Iacovitti, L. (2006). Tyrosine hydroxylase gene regulation in human neuronal progenitor cells does not depend on Nurr1 as in the murine and rat systems. *Journal of cellular physiology*, *207*(1), 49-57.

Joober, R., Toulouse, A., Benkelfat, C., Lal, S., Bloom, D., Labelle, A., ... & Rouleau, G. A. (2000). DRD3 and DAT1 genes in schizophrenia: an association study. *Journal of psychiatric research*, *34*(4), 285-291.

Kadkhodaei, B., Ito, T., Joodmardi, E., Mattsson, B., Rouillard, C., Carta, M., ... & Chambon, P. (2009). Nurr1 is required for maintenance of maturing and adult midbrain dopamine neurons. *The Journal of Neuroscience*, *29*(50), 15923-15932.

Kessler, M. A., Yang, M., Gollomp, K. L., Jin, H., & Iacovitti, L. (2003). The human tyrosine hydroxylase gene promoter. *Molecular brain research*, *112*(1), 8-23.

Khan, Z. U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A., & De La Calle, A. (1998). Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain.

Journal of Comparative Neurology, *402*(3), 353-371.

Khorasanizadeh, S., & Rastinejad, F. (2001). Nuclear-receptor interactions on DNA-response elements. *Trends in biochemical sciences*, *26*(6), 384-390.

Kim, H. G., Lee, J. S., Han, J. M., Lee, J. S., Choi, M. K., Son, S. W., ... & Son, C. G. (2013). Myelophil attenuates brain oxidative damage by modulating the hypothalamus-pituitary-adrenal (HPA) axis in a chronic cold-stress mouse model. *Journal of ethnopharmacology*, *148*(2), 505-514.

Kim, T. E., Seo, J. S., Yang, J. W., Kim, M. W., Kausar, R., Joe, E., ... & Lee, M. A. (2013). Nurr1 represses tyrosine hydroxylase expression via SIRT1 in human neural stem cells. *PloS one*, *8*(8), e71469.

Kochanek, K. D., Murphy, S. L., Xu, J., & Tejada-Vera, B. (2016). Deaths: Final Data for 2014. *National Vital Statistics Reports, 65*(4). Retrieved October 31, 2016, from http://www.cdc.gov/nchs/data/nvsr/nvsr65/nvsr65_04.pdf

Kovalovsky, D., Refojo, D., Liberman, A. C., Hochbaum, D., Pereda, M. P., Coso, O. A., ... & Arzt, E. (2002). Activation and induction of NUR77/NURR1 in corticotrophs by CRH/cAMP: involvement of calcium, protein kinase A, and MAPK pathways. *Molecular Endocrinology*, *16*(7), 1638-1651.

Kręzel, W., Ghyselinck, N., Samad, T. A., Dupé, V., Kastner, P., Borrelli, E., & Chambon, P. "Impaired locomotion and dopamine signaling in retinoid receptor mutant mice." *Science* 279.5352 (1998): 863-867.

Kvetňanský, R., Culman, J., Tigranian, R., Torda, T., Opršalová, Z., & Macho, L. (1984). Catecholamines in Discrete Brain Areas of Rats Exposed to Weightlessness During

Space Flights. In *Stress, the Role of Catecholamines and Other Neurotransmitters: Proceedings of the Third International Symposium on Catecholamines and Other Neurotransmitters in Stress, Smolenice Castle, Czechoslovakia, June 7-12, 1983* (Vol. 1, p. 1011). CRC Press.

Kvetňanský, R., Lu, X., & Ziegler, M. G. (2013). Stress-triggered changes in peripheral catecholaminergic systems. *Adv Pharmacol*, *68*, 359-397.

Langmead, B., Trapnell, C., Pop, M., & Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome biology*, *10*(3), 1.

Liu, X., Serova, L., Kvetňanský, R., & Sabban, E. L. (2008). Identifying the stress transcriptome in the adrenal medulla following acute and repeated immobilization. *Annals of the New York Academy of Sciences*, *1148*(1), 1-28.

McEvoy, A. N., Murphy, E. A., Ponnio, T., Conneely, O. M., Bresnihan, B., FitzGerald, O., & Murphy, E. P. (2002). Activation of nuclear orphan receptor NURR1 transcription by NF-κB and cyclic adenosine 5′-monophosphate response element-binding protein in rheumatoid arthritis synovial tissue. *The Journal of Immunology*, *168*(6), 2979-2987.

Meador-Woodruff, J. H., Grandy, D. K., Van Tol, H. H., Damask, S. P., Little, K. Y., Civelli, O., & Watson, S. J. (1994). Dopamine receptor gene expression in the human medial temporal lobe. *Neuropsychopharmacology*, *10*(4), 239-248.

Meiser, J., Weindl, D., & Hiller, K. (2013). Complexity of dopamine metabolism. *Cell Communication and Signaling*, *11*(1), 1.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of neuroscience methods*, *11*(1), 47-60.

Mulligan, M.K., Mozhui, K., Prins, P., and Williams, R.W. (2016). GeneNetwork – A toolbox for systems genetics. In Schughart, K. and Williams, R.W. (Eds.), *Systems Genetics*, Methods in Molecular Biology. New York City: Humana Press, in press.

Park, J. S., Yang, H. N., Woo, D. G., Jeon, S. Y., Do, H. J., Huh, S. H., ... & Park, K. H. (2012). Exogenous Nurr1 gene expression in electrically-stimulated human MSCs and the induction of neurogenesis. *Biomaterials*, *33*(29), 7300-7308.

Patki, G., Atrooz, F., Alkadhi, I., Solanki, N., & Salim, S. (2015). High aggression in rats is associated with elevated stress, anxiety-like behavior, and altered catecholamine content in the brain. *Neuroscience letters*, *584*, 308-313.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral neuroscience*, *106*(2), 274.

Rasheed, N., Ahmad, A., Pandey, C. P., Chaturvedi, R. K., Lohani, M., & Palit, G. (2010). Differential response of central dopaminergic system in acute and chronic unpredictable stress models in rats. *Neurochemical research*, *35*(1), 22-32.

Rojas, P., Joodmardi, E., Hong, Y., Perlmann, T., & Ögren, S. O. (2007). Adult mice with reduced Nurr1 expression: an animal model for schizophrenia. *Molecular psychiatry*, *12*(8), 756-766.

Rondou, P., Haegeman, G., & Van Craenenbroeck, K. (2010). The dopamine D4 receptor: biochemical and signalling properties. *Cellular and molecular life sciences*, *67*(12),

1971-1986.

Rowe, D. C., Stever, C., Gard, J. M., Cleveland, H. H., Sanders, M. L., Abramowitz, A., ... & Waldman, I. D. (1998). The relation of the dopamine transporter gene (DAT1) to symptoms of internalizing disorders in children. *Behavior genetics*, *28*(3), 215-225.

Sabban, E. L., & Kvetňanský, R. (2001). Stress-triggered activation of gene expression in catecholaminergic systems: dynamics of transcriptional events. *Trends in neurosciences*, *24*(2), 91-98.

Sacchetti, P., Mitchell, T. R., Granneman, J. G., & Bannon, M. J. (2001). Nurr1 enhances transcription of the human dopamine transporter gene through a novel mechanism. *Journal of neurochemistry*, *76*(5), 1565-1572.

Sakurada, K., Ohshima-Sakurada, M., Palmer, T. D., & Gage, F. H. (1999). Nurr1, an orphan nuclear receptor, is a transcriptional activator of endogenous tyrosine hydroxylase in neural progenitor cells derived from the adult brain. *Development*, *126*(18), 4017-4026.

Scheggi, S., Leggio, B., Masi, F., Grappi, S., Gambarana, C., Nanni, G., ... & De Montis, M. G. (2002). Selective modifications in the nucleus accumbens of dopamine synaptic transmission in rats exposed to chronic stress. *Journal of neurochemistry*, *83*(4), 895-903.

Schultz, W. (2007). Multiple dopamine functions at different time courses. *Annu. Rev. Neurosci.*, *30*, 259-288.

Serretti, A., Macciardi, F., Cusin, C., Verga, M., Pedrini, S., & Smeraldi, E. (1998).

Tyrosine hydroxylase gene in linkage disequilibrium with mood disorders. *Molecular psychiatry*, *3*(2), 169-174.

Sidhu, A. (1998). Coupling of D1 and D5 dopamine receptors to multiple G proteins. *Molecular neurobiology*, *16*(2), 125-134.

Thibaut, F., Ribeyre, J. M., Dourmap, N., Meloni, R., Laurent, C., Campion, D., ... & Petit, M. (1997). Association of DNA polymorphism in the first intron of the tyrosine hydroxylase gene with disturbances of the catecholaminergic system in schizophrenia. *Schizophrenia research*, *23*(3), 259-264.

Torii, T., Kawarai, T., Nakamura, S., & Kawakami, H. (1999). Organization of the human orphan nuclear receptor Nurr1 gene. *Gene*, *230*(2), 225-232.

Trapnell, C., Pachter, L., & Salzberg, S. L. (2009). TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics*, *25*(9), 1105-1111.

Waarde, A. V., Vaalburg, W., Doze, P., Bosker, F. J., & Elsinga, P. H. (2004). PET imaging of beta-adrenoceptors in human brain: a realistic goal or a mirage? *Current pharmaceutical design*, *10*(13), 1519-1536.

Wang, Z., Benoit, G., Liu, J., Prasad, S., Aarnisalo, P., Liu, X., ... & Perlmann, T. (2003). Structure and function of Nurr1 identifies a class of ligand-independent nuclear receptors. *Nature*, *423*(6939), 555-560.

Wärnmark, A., Treuter, E., Wright, A. P., & Gustafsson, J. A. (2003). Activation functions 1 and 2 of nuclear receptors: molecular strategies for transcriptional activation. *Molecular endocrinology*, *17*(10), 1901-1909.

Zhu, M. Y., Juorio, A. V., Paterson, I. A., & Boulton, A. A. (1993). Regulation of striatal

aromatic L-amino acid decarboxylase: effects of blockade or activation of dopamine receptors. *European journal of pharmacology*, *238*(2-3), 157-164.

Zhu, M. Y., Klimek, V., Dilley, G. E., Haycock, J. W., Stockmeier, C., Overholser, J. C., ... & Ordway, G. A. (1999). Elevated levels of tyrosine hydroxylase in the locus coeruleus in major depression. *Biological psychiatry*, *46*(9), 1275-1286.

Zucker, M., Weizman, A., & Rehavi, M. (2005). Repeated swim stress leads to down-regulation of vesicular monoamine transporter 2 in rat brain nucleus accumbens and striatum. *European neuropsychopharmacology*, *15*(2), 199-201.